THE INHERITANCE OF COMPONENTS OF RESISTANCE TO BACTERIAL LEAF SPOT OF PEPPER (*Capsicum annuum* L.)

BY

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The efficient discharge of responsibilities in breeding disease resistant pepper is dedicated to

ALLYN AUSTIN COOK

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TABLE OF CONTENTS

LIST OF TABLES

Tables Page

Tables Page

Tables Page

LIST OF FIGURES

Figures Page

Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

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Inheritance of resistance to bacterial leaf spot of pepper (*Capsicum annuum* L.) incited by *Xanthomanas campestris* pv. *vesicatoria* (Doidge) Dye was evaluated in progenies of resistant germplasm crossed with susceptible bell pepper.

The basis for designating pathogenic races was avirulence on plants with genes derived from PI 271322. Race 1 induced a hypersensitive response (HR) in plants with Bs_3, race 2 induced HR in plants with Bs_1, and race 3 did not induce HR. Race 3 occurred frequently in laboratory cultures of races 1 and 2.

Eighteen PI and 6 breeding lines had genetic resistance to races 1, 2, and 3. In addition, plants of PI 163189 had gene Bs_1, and PI 271322 had Bs_1 and Bs_3.

Resistance to race 1 and to race 2 was detected after injections of leaves of plants with Bs_1 and Bs_3 with high concentrations of inocula ($>10^8$ cells ml^{-1}). Collapse of tissues occurred within 24 hours with

race 1 and 12 hours with race 2. Concomitantly, electrolyte losses occurred and bacterial multiplication was inhibited. Both genes were incompletely dominant in heterozygotes of PI 271322 crossed with susceptible pepper.

The gene $\underline{Bs_2}$ in cv. Florida XVR 3-25 controlled HR to both races 1 and 3. Genes $\underline{Bs_1}$, $\underline{Bs_2}$, and $\underline{Bs_3}$ segregated independently in crosses.

Resistance to race 3 and concomitantly to races 1 and 2 was identified after injections of leaves with low concentrations of inocula (2×10^3 cells ml^{-1}). In resistant plants, lesions were fewer and always smaller than in susceptible plants. Lesion diameter and field resistance to race 3 were correlated (r=0.99).

Monogenic additive-gene action controlled resistances to race 3 in PI 163189, 246331, and 271322. Lesion diameters in heterozygotes varied around mid-parent values. Parental values varied according to the bacterial isolates used for inoculation. Lesion numbers in heterozygotes were few with weak isolates, but equalled the susceptible parent with aggressive isolates. The proposed symbol for this gene in PI 271322 is $\underline{Bs_4}$. Gene $\underline{Bs_4}$ segregated independently of $\underline{Bs_1}$ and $\underline{Bs_3}$ in crosses. Bell pepper stocks with all four genes were produced.

CHAPTER 1
INTRODUCTION

Bacterial leaf spot, incited by the gram negative, motile bacterium, Xanthomonas campestris pv. vesicatoria (Doidge, 1920), Dye 1978 (hereafter designated Xcv), is the most destructive leaf disease of bell pepper (Capsicum annuum L.) in warm humid environments. Disease severity varies with the time of primary infection and may extend from loss of a few lower leaves to complete defoliation of susceptible cultivars. Yield loss accrues from failure to set fruit, and from undersized and sunburnt fruit. Refoliation and additional flowering can occur but fruit set are later maturing and smaller. Quantitative yield data to assess the effects of disease are uncommon, but, overall, a loss of 10% to production in the state of Florida may be average. This translates to a fruit value of $7.5 million on 1983-84 figures (Hochmuth and Maynard, 1985).

The bacterial spot organism is endemic in warm humid environments. A prime source of inoculum is overwintering and oversummering volunteer peppers and tomatoes (Krupka and Crossan, 1956; Person, 1965; Pohronezny, 1984). Inoculum dispersal is aided by a continuum over time of pepper and tomato crops in many regions, and frequent wind driven rains (Vakili, 1967). Disease control by sanitation has been insufficient to eliminate the need for chemical and genetic control of Xcv. Chemical control relies on frequent sprays with copper and mancozeb mixture (Marco and Stall, 1983). However, the most strictly applied spray regime is expected to be less effective than genetic resistance to the

pathogen. Resistant cultivars are the more energy efficient method of disease control, and they contribute to major savings in crop production costs.

Several releases of bell pepper (Cook, 1984; Cook et al., 1976; Cook et al., 1977) were selected in part for the dominant genes for hypersensitive resistance, $\underline{Bs_1}$ (Cook and Stall, 1963), and $\underline{Bs_2}$ (Cook and Guevara, 1984). Race 2 of the strain of Xcv pathogenic on pepper (Cook and Stall, 1969) induced the hypersensitive reaction (HR) in plants with $\underline{Bs_1}$, but virulence of race 1 was not affected by this gene (Cook and Stall, 1968; 1969; 1982). The gene $\underline{Bs_2}$ controlled hypersensitivity to race 1 (Cook and Guevara, 1982; 1984), and isolates virulent on plants with $\underline{Bs_1}$ and $\underline{Bs_2}$ were not observed in limited testing. Recently, Kim and Hartmann (1985) reported a third gene $\underline{Bs_3}$, which also controls HR to isolates of race 1. The relation between $\underline{Bs_2}$ and $\underline{Bs_3}$ is unknown, and Kim and Hartmann (1985) assumed they were different loci. Releases of bell pepper with $\underline{Bs_3}$ have not been made, and testing for pathogenic variability in relation to $\underline{Bs_3}$ was not extensive (Kim and Hartmann, 1985).

Race 2 of Xcv occurs with race 1 in Florida but has been detected in few other pepper producing areas of the world (Cook and Stall, 1982; F.R.J.B. Reifschneider, 1985, personal communication to R.E. Stall). Nevertheless, $\underline{Bs_1}$ was useful in delaying disease progress in Florida (Dahlbeck et al., 1979). The $\underline{Bs_3}$ gene appeared to contribute much to low disease severity in field plantings infected with race 1 (Kim and

Hartmann, 1985). Resistance to race 1 controlled by both $\underline{Bs_2}$ and $\underline{Bs_3}$ together, if indeed the genes are different, may be long lasting.

Stable resistance to Xcv is found in many pepper plant introductions (PI lines) (Adamson and Sowell, 1983; Borchers, 1965; Dempsey, 1953; Hibberd et al., 1979; Hibberd and Gillespie, 1982a; Kim, 1983; Sowell and Dempsey, 1977; Stall, 1981). Most sources lack hypersensitive responses to races 1 and 2 (Cook and Stall, 1969; Kim, 1983; Stall, 1981). Nevertheless, disease severity was consistently very low on these lines. No releases of bell pepper have yet been made using these sources of resistance. Borchers (1965) used several PI lines, and made progress by selecting among naturally infected plantings of inbred backcrosses. Breeding was discontinued, however, and seed is no longer available (E.A. Borchers, 1978, personal communication). Progress has been slow in other breeding programs (Adamson and Sowell, 1983; Hibberd and Gillespie, 1982a). There are several reasons for this:

1. Some plant breeders are unwilling to use race specific genes for fear of instability of resistance (Adamson and Sowell, 1983; Hibberd and Gillespie, 1982a).

2. Inoculation techniques that are frequently used fail to introduce the bacteria directly into the mesophyll of leaves, which is the site of bacterial activity (Stall and Cook, 1966). Using these techniques, consistent, quantifiable data are not reliable and estimates of heritability of resistance are reduced accordingly (Adamson and Sowell, 1983; Hibberd and Gillespie, 1982a).

3. Genetics of resistance is often assumed to be complex and quantitative.

4. Guiding plant pathologists may regard the hypersensitive reaction as an artifact of the unnaturally high inoculum density used to detect it most easily (Klement et al., 1964). This has the effect of enforcing a selection task more difficult than necessary on unsuspecting plant breeders.

5. More than one resistance mechanism occurring in single plants may not be recognized unless appropriate inoculation techniques are used. Inheritance studies may be confounded unless variability in host responses is recognized (Adamson and Sowell, 1983; Kim and Hartmann, 1985; Stall, 1981).

It is important that genes responsible for stable, effective resistance be identified and characterized. Knowledge of efficient selection methods will simultaneously accrue. Research was undertaken with these views in mind. Particular attention was given to two aspects. These were allelism and characterization of known genes for hypersensitive resistance, and the inheritance of genes for nonhypersensitive resistance. As a consequence, efficient selection procedures were defined, and resistance genes were moved from commercially unadapted germplasm to bell pepper.

This research is comprised of a series of related topics and experiments. They are described in several discreet chapters. The reader is forewarned that this was achieved at the expense of some repetition.

CHAPTER 2
REVIEW OF LITERATURE

Apparently healthy plants may harbor a wide range of bacteria (Leben, 1974). These gain entry through natural plant openings, such as stomata and hydathodes, and through wounds such as those induced by wind and rain driven sand particles (Goodman, 1982; Vakili, 1967). The bacterium, after entering, may induce the following to happen: If the bacterium is a saprophyte, bacteria may multiply little if at all, and no host tissue damage occurs. If the bacterium is a parasite, numbers may increase over a short time span (often measured in hours) and subsequently stabilize or decline slowly with no visible damage occurring. This sequence of events is associated with localized hypersensitive death of host cells. A second reaction type may occur with parasitic bacteria in that populations continue to increase to high levels, which is associated with extensive and visible host tissue necrosis. This is the susceptible reaction.

Resistance to a parasitic bacterium is usually observed as lower disease severity consistent with lower bacterial populations. Low populations of bacteria are often associated with hypersensitivity, but other forms of resistance may occur which are not typically hypersensitive (Klement, 1982). Nevertheless, hypersensitivity appears to be a general resistance mechanism in plants to bacterial parasites (Klement, 1982).

Hypersensitive Resistance

Hypersensitivity in plants is commonly observed after inoculation of species of pseudomonads and xanthomonads among phytopathogenic bacteria. Its development in plants after inoculation has been useful both as an aid to identification of bacterial species (Lelliott et al., 1966) and determination of host ranges (Klement, 1982). A hypersensitive reaction (HR) occurs in plant species which normally are not susceptible to the bacterial species. In all examples studied, HR was found associated with vastly fewer bacteria per unit area of leaf than occurring in compatible, susceptible reactions (Allington and Chamberlain, 1949; Ercolani and Crosse, 1966; Klement et al., 1964; Lyon and Wood, 1976; Roebuck et al., 1978).

As a consequence, HR was sought also in pathosystems involving normally compatible bacteria, and many examples of its occurrence can now be cited (Brinkerhoff et al., 1984; Cook, 1973; Cook and Guevara, 1984; Cook and Stall, 1968, 1969; Fallik et al., 1984; Gitaitis, 1983; Kim and Hartmann, 1985; Lawson and Summers, 1984; Long et al., 1985; Mukherjee et al., 1966; Patel, 1982; Smith and Mansfield, 1981; Walker and Patel, 1964). In those cases studied closely, HR was found to function in the same manner as that occurring between parasitic bacteria and nonhost species (Al-Mousawi et al., 1982; Cook and Stall, 1968; Lyon and Wood, 1976; Roebuck et al., 1978; Stall and Cook, 1966); that is, host cell metabolism was severely disrupted and death soon followed. Concomitantly, bacterial populations were much lower than in susceptible reactions.

The HR is inducible (Meadows and Stall, 1981; Klement, 1982). Cell-to-cell contact between living host and bacterial cells is required

for induction of HR but not the susceptible reaction (Lyon and Woods, 1976; Stall and Cook, 1979; Young, 1974). However, Keen and Holliday (1982) and Long et al. (1985) argued against the requirement for cell-to-cell contact. A specific recognition event is presumed to occur during this phase (Keen, 1982) but its nature is unknown (Staskawicz et al., 1984). The recognition is under genetic control (Stall, 1985; Staskawicz et al., 1984) according to the model of a locus in the pathogen interacting with a complementary locus in the host (Flor, 1955). Induction time for HR varies in part with inoculum concentration. In some pathosystems a longer time is required for symptoms of HR to occur when low concentrations of bacteria are used than with high concentrations (Essenberg et al., 1979). This implies a minimum concentration of interacting gene products is essential for HR to proceed. Length of the induction period is usually measured in plant tissues inoculated with high concentrations of bacteria. In such tests, induction time varies between approximately 1 and 5 h after inoculation after which HR usually proceeds irreversibly (Klement, 1982).

Once HR is induced, host cell collapse follows a latent period of variable length, during which biochemical events leading to cell collapse are presumed to occur (Klement, 1982). The cell-collapse phase of HR occurs with an irreversible breakdown of membranes and plastids, and the mixing of cytoplasmic and vacuolar contents (Al-Mousawi et al., 1982; Cook and Stall, 1968; Lyon and Wood, 1976; Roebuck et al., 1978; Stall and Cook, 1966). Inoculated leaf tissues lose turgor as metabolism is disrupted, and loss of electrolytes increases greatly (Cook and Stall, 1968; Cook and Guevara, 1984). Electrolyte losses also occur

from tissues in the susceptible reaction but the rate of increase is slow and gradual in comparison with HR-induced losses. Concomitantly, bacteria cease multiplying (Klement et al., 1964), and their numbers may progressively decline over time (Cook and Guevara, 1984). Host cells may accumulate large amounts of antibiotic phytoalexins prior to tissue collapse (Long et al., 1985). Release of these and acid vacuolar contents during cell collapse imposes a localized environment which inhibits further bacterial multiplication (Klement et al., 1964; Long et al., 1985), and bacteria may die.

High inoculum concentration is vital to detect HR easily. All host cells in the inoculated tissue collapse in response to inoculum containing 10^7 to 10^8 cells ml^{-1}. Genes in pepper for hypersensitivity were not identified and characterized as such until their phenotypes were clearly visualized by using such high concentrations (Cook and Guevara, 1984; Cook and Stall, 1963; Kim and Hartmann, 1985; Stall and Cook, 1966). Necrotic hypersensitive flecks will develop occasionally in leaves that are surface sprayed with the same high concentration of bacteria. Bacteria may have entered through some stomata in sufficient numbers for isolated visible HR-necroses to develop (Cook and Stall, 1963). When leaves are infiltrated with low concentrations of bacteria, occasional small necrotic flecks may result from limited bacterial multiplication (Essenberg et al., 1979), but visible necroses often do not occur.

Hypersensitivity appears to be under genetic control. The host genes that are responsible for its induction in cultivars or plant introduction (PI) lines of a normally susceptible species can be explored by genetic crosses. However, inheritance of surprisingly

few reactions has been examined (Long et al., 1985), and much of the knowledge of HR stems from the work of Cook and Stall with pepper (Capsicum annuum L.) and Xanthomonas campestris pv. vesicatoria (Doidge, 1920), Dye 1978 (hereafter designated as Xcv).

Variation in the Pepper--Xanthomonas campestris pv. vesicatoria System

Three dominant genes for HR to Xcv have been described in pepper. Subscripts following gene designation follow the terminology of Lippert et al. (1965). The first gene, Bs_1, was found in a heterogenous PI line (Cook and Stall, 1963). Hypersensitivity induced by Bs_1 was confirmed by observations of ultrastructure (Sasser, Stall, and Cook, 1968), leakage of ions from inoculated tissue (Cook and Stall, 1968), and changes in bacterial populations in vivo (Cook, 1973; Stall and Cook, 1966). The gene was incorporated by standard backcrossing with selection into releases of several bell peppers (Cook, 1984; A.A. Cook, 1979 and 1982, personal communications).

From an early stage of selection for Bs_1 in segregating progeny, variants of Xcv were noted which induced a susceptible reaction rather than HR. Two races were clearly differentiated by plants with this gene. Race 1 induced the susceptible reaction, and race 2 induced HR. Near isogenic lines of pepper, with the difference based on Bs_1, were produced. These have found extensive use in studies of the physiology of HR (Cook, 1973; Cook and Stall, 1968, 1971; Stall et al., 1981), induction of HR (Meadows and Stall, 1981; Stall et al., 1974; Stall and Cook, 1979), racial instability in Xcv (Dahlbeck and Stall, 1979; Dahlbeck et al., 1979; Stall et al., 1984), influence of Bs_1 on

disease progress and pathogenesis (Dahlbeck et al., 1979; Stall, 1985) and the distribution in nature of races of Xcv (Cook and Stall, 1982). The HR to race 2 was observed in several other PI lines (Cook and Stall, 1969) but allelism of genes was not tested.

Races 1 and 2 of the pepper strain of Xcv occur in Florida's pepper producing areas, but race 1 and not race 2 appears to predominate in other regions of the world (Cook and Stall, 1982). Resistance to race 1 is essential for control of bacterial spot. Cook reported a dominant gene, $\underline{Bs_2}$, for HR to race 1, which he found in a plant introduction of C. chacoense L. (Cook and Guevara, 1982; 1984). A recent bell pepper release (Cook, 1984) was produced by selecting for $\underline{Bs_2}$ in recurrent backcrosses to a cultivar with $\underline{Bs_1}$. The HR controlled by $\underline{Bs_2}$ differed symptomatically from that controlled by $\underline{Bs_1}$ (Cook and Guevara, 1982), and Cook (1977) suggested linkage of resistance to both races 1 and 2 occurred in the C. chacoense line. Kim and Hartmann (1985) assumed that $\underline{Bs_2}$ controlled HR to both races 1 and 2, but their assumption was based on a mis-interpretation of the Cook and Guevara (1982) abstract. Isolates of race 1 virulent on plants with $\underline{Bs_2}$ were not detected in limited testing (A.A. Cook, 1984, personal communication). The extensive studies of HR controlled by $\underline{Bs_1}$ have not been repeated with $\underline{Bs_2}$.

Recently Kim and Hartmann (1985) reported another dominant gene, $\underline{Bs_3}$, in C. annuum that controls HR to race 1 of the pepper strain. Hypersensitivity induced by this gene was not characterized. The few isolates tested induced HR in plants with $\underline{Bs_3}$. The genetic relationship between $\underline{Bs_2}$ and $\underline{Bs_3}$ is unknown.

Concomitantly with this work, other isolates of Xcv were noted that induced hypersensitivity in all tested peppers, irrespective of the presence of Bs_1, Bs_2, or Bs_3. Susceptibility occurred in tomato, and these isolates were designated as the tomato strain of Xcv to distinguish them from races 1 and 2 of the pepper strain (Cook and Stall, 1969). The HR to the tomato strain differed in several ways from HR of plants with Bs_1 to race 2 of the pepper strain (Cook, 1973). The HR to the tomato strain was symptomatically different and was visibly slow in developing. The loss of ions from leaf tissue infiltrated with the tomato strain was intermediate to losses occurring with HR in plants with Bs_1 inoculated with race 2 and the susceptible reaction. Bacterial populations were correspondingly higher with the tomato strain than with race 2 in plants with Bs_1, but lower than in the fully susceptible reaction. Several environmental variables (light, darkness, and Ca $(NO_3)_2$-infiltrated leaves) had contrasting influences on the two forms of HR. These comparisons are evidence that HR induced by distinct pathotypes may involve quite different biological processes. One process is inherited as a dominant, single-gene trait, but genetic variability could not be determined for the other.

The tomato strain of Xcv is a variant based on the reaction of two host species. It may more appropriately be regarded as a pathovar of Xanthomonas campestris distinct from Xcv in that pepper does not appear to be its natural host. Similarly, Kimura et al. (1972) mentioned Brazilian isolates of Xcv which induced HR in tomato and susceptibility in pepper irrespective of the presence of Bs_1 (F.R.J.B. Reifschneider, 1985, personal communication to R.E. Stall). Two profitable areas for

future research may involve identifying the genetic factors in pepper and tomato responsible for these two differential host-pathogen interactions, and transferring them reciprocally between plant species. Nevertheless, Capsicum is a diverse genus which may yield many other forms of HR to Xcv that are transferrable by crossing within C. annuum.

Hypersensitivity in Other Pathosystems

The HR in the pepper and Xcv system is a model because of the extent of its characterization and usage. Other pathosystems have been treated to varying extents. The HR in soybean to the bacterial blight organism has also been intensively studied (Keen, 1982; Staskawicz et al., 1984; Long et al., 1985) but with relatively little effort directed to inheritance of resistance (Mukherjee et al., 1966). Patel (1982) reported two race-specific dominant genes in cowpea for HR to bacterial pustule. The reaction to one race developed consistently slower by several hours than HR to the other race in the same plant, but both appeared typical of HR. Genes in cotton for hypersensitivity to bacterial blight also are dominant (Brinkerhoff et al., 1984), appear to be race specific (Bayles and Johnson, 1985), and have been recombined in useful releases. The races of the blight bacterium have not been characterized extensively. Dominant, race-specific genes for HR in several other pathosystems have found commercial use (Walker and Patel, 1964; Taylor et al., 1978; Innes et al., 1984; Fallik et al., 1984; Lawson and Summers, 1984). Those and others have been characterized to varying extents (Ercolani and Crosse, 1966; Lyon and Woods, 1976; Roebuck et al., 1978; Young, 1974). Slow developing or atypical hypersensitivity has been reported also in several pathosystems besides pepper with Xcv. Both Patel (1982) and Gitaitis (1983) reported that

isolates of the bacterial pustule organism in cowpea induced a slow developing atypical HR. Ersek and Hevesi (1983), Jones and Fett (1985), and Long et al. (1985) reported intermediate, that is, slow developing HR in soybean inoculated with the bacterial blight organism, and Smith and Mansfield (1981) characterized some effects of slow HR in oats to the halo blight bacterium. Cucumber reacted to the angular leaf spot bacterium with atypical HR (Dessert et al., 1982). In all cases, atypical HR represented a high degree of resistance to the pathogen and resembled the description of HR in pepper to the tomato strain of Xcv (Cook, 1973).

Commonly, typical HR is race-specific (Keen, 1982). This may not be so for atypical or slow developing HR (Cook, 1973). Patel (1982) preferred to select for the slow developing HR since it appeared functional against all races. However, in the few cases studied, these resistances were recessively inherited in contrast to dominance of typical, race-specific HR (Dessert et al., 1982; Jones and Scott, 1985; Patel, 1982). Consequently, inbred backcross progenies may be necessary for selection purposes. This effectively doubles the number of generations required in breeding for these resistances. Despite these acknowledged variations, hypersensitivity is considered a qualitative trait of major effect. It is functional at widely different plant maturities (Laurence and Kennedy, 1974; Bayles and Johnson, 1985; Cook and Stall, 1968), and inoculum concentrations (Essenberg et al., 1979; Turner and Novacky, 1974), and is significantly influenced only by extremes of environmental conditions (Cook, 1973; Stall and Cook, 1979; Keen, 1982; Klement, 1982).

Nonhypersensitive Resistances to Xcv in Pepper

Genes for HR in pepper were found in a few PI lines only after extensive testing of germplasm derived mainly from the Indian subcontinent (Cook, 1977; Cook and Stall, 1963; Kim and Hartmann, 1985; Sowell, 1980). Many other PI lines were found which also are highly resistant to Xcv (Borchers, 1965; Dempsey, 1953; Greenleaf, 1960; Hibberd et al., 1979; Hibberd and Gillespie, 1982a; Kim, 1983; Sowell, 1960; Sowell and Dempsey, 1977). These lines either lacked HR entirely or were heterogeneous for HR to races 1 or 2 (Cook and Stall, 1969; Kim and Hartmann, 1985). No PI line, with the possible exception of the above mentioned *C. chacoense* line with Bs_2, was observed with HR to both races. Several nonhypersensitively resistant PI lines have been used in breeding. Borchers (1965) and Hibberd and Gillespie (1982a) selected resistant segregates from among field-grown, naturally infected F_3 and inbred backcross progenies. By implication, this resistance was not inherited as a dominant trait, but as a recessive or incomplete recessive (Hibberd and Gillespie, 1982a). Two independent resistance genes were detected only in F_3 progenies of crosses between resistant PI lines and susceptible bell pepper (Adamson and Sowell, 1983).

Substantial progress was made in breeding for nonhypersensitive resistance, but releases of bell pepper have not eventuated. Several reasons contributed to slow progress. Selection in field-planted progenies was, not unexpectedly, effective only in environments highly conducive to disease spread (Hibberd and Gillespie, 1982a). Similarly, selection was ineffective in some greenhouse-grown experiments of short duration. In these, bacteria were applied only superficially to leaves

(Adamson and Sowell, 1983). The inoculum dosage actually received at the mesophyll where bacteria multiply cannot be standardized in such tests. Heritability of resistance was low.

These problems were circumvented by examining components of resistance (Stall, 1981; Stall et al., 1982b). Initial numbers of bacteria per unit of leaf area are directly proportional to their concentration in inoculum used to infiltrate leaves (Klement et al., 1964; Essenberg et al., 1979; Dahlbeck and Stall, 1979; Turner and Novacky, 1974; Stall et al., 1982b). Bacterial colonies develop in the mesophyll from single bacteria (Essenberg et al., 1979). These may grow to form lesions, and their frequency per unit area of leaf reflects resistance (Stall, 1981; Stall et al., 1982b). Nonhypersensitive resistance in pepper assessed by this method was recessive but continuously distributed (Stall, 1981). Resistance selected in an F_2 progeny was highly heritable, and F_3 lines were identified which were homozygous and and homogeneous (R.E. Stall, 1982, personal communication). Nonhypersensitive resistance was more clearly and effectively identified by this technique than any other (compare Cook and Stall, 1969; and Stall, 1981). Replication within plants was necessary to quantify resistance. Seedlings were therefore required to be at a more mature stage than was necessary to identify qualitative genes for HR, and the workload per selection cycle increased correspondingly.

Nonhypersensitive Resistance in Other Pathosystems

Hypersensitivity is not known or recognized in some other pathosystems involving xanthomonad bacteria, for example common blight of bean and bacterial blight of rice. Pathogenic races sensu Cook and Stall (1969) are not known in these cases. Instead isolates may be

clustered into groups by their degree of virulence on standard sets of cultivars (Mew et al., 1982; Schuster and Coyne, 1971, 1975). The degree of resistance varies with the bacterial isolates, test environment, stage of plant growth, and the plant tissue (Coyne et al., 1973; Coyne and Schuster, 1974a, 1974b; Valladares-Sanchez et al., 1979; Sidhu and Khush, 1978; Yoshimura et al., 1984).

Inheritance of these resistances may be additive, dominant, or recessive. Dominance often is incomplete. Resistance is quantitative in all cases. Relatively discreet classes of resistant and susceptible plants in segregating populations were observed with some systems (Yoshimura et al., 1983, 1984), but not others (Coyne and Schuster, 1979; Coyne et al., 1973). More accurate classification of single plants occurred where at least one important component of resistance was reliably measured. For example, the bacterial blight pathogen was applied directly to exposed rice leaf tissue by damaging leaves. Lesion length developed in a given time from these inoculation sites accurately reflected resistance (Yoshimura et al., 1984). Diffuse chlorosis in other pathosystems may preclude accurate measurements of disease development (Coyne and Schuster, 1974b). In that event, quantitative resistance was better assessed in terms of quantitative genetics (Valladares-Sanchez, 1979). Additive gene dosage effects appear to be important in these examples of nonhypersensitive resistances (Valladares-Sanchez, 1979). Heterozygotes but not necessarily homozygotes may interact strongly with many environmental factors (Sidhu and Khush, 1978; Mew et al., 1982). This strongly implies an effect of gene dosage on degree of resistance.

There are several similarities between nonhypersensitive resistance in pepper to Xcv and other pathosystems. Nonhypersensitive resistance

may be durable, but the degree of resistance may vary between host lines (Mew et al., 1982; Schuster and Coyne, 1975; Sowell, 1960; Sowell and Dempsey, 1977). Bacterial isolates also may vary in their degree of virulence (Cook, 1973; Mew et al., 1982). Nonhypersensitive resistance to a broad range of bacterial isolates may be controlled by one or a few genetic loci in the host (Brinkerhoff et al., 1984; Cook and Stall, 1969; Patel, 1982; Sowell and Dempsey, 1977; Stall, 1981; Yoshimura et al., 1984). More than one locus may exist in any one host (Adamson and Sowell, 1983; Patel, 1982; Yoshimura et al., 1984). The degree of resistance controlled by these loci in heterozygotes may vary with the test conditions (Adamson and Sowell, 1983; Hibberd and Gillespie, 1982a; Sidhu and Kush, 1978; Valladarez-Sanchez et al., 1979; Yoshimura et al., 1984) so that inbred progenies may be necessary to identify resistant homozygotes (Adamson and Sowell, 1983; Brinkerhoff et al., 1984; Patel, 1982; Stall, 1981). Accurate quantification of resistance requires measuring its components (Mew et al., 1982; Stall, 1981; Yoshimura et al., 1984), and few nonhypersensitive resistances have been well characterized.

Nonhypersensitive resistances have found greatest use in supplementing race specific genes for HR (Fallik et al., 1984; Innes et al., 1984; Lawson and Summers, 1984; Patel, 1982; Walker and Patel, 1964). It is probable that genes for nonhypersensitive resistance are fundamentally of greater importance than genes for race-specific HR, and that the latter should be used to supplement the former. Finally, the distinguishing difference in physiology of HR in its various forms and nonhypersensitive resistance has never been established.

Summary

The following conclusions may be drawn. Breeding for resistance to bacterial leaf spot in pepper is necessary. Genes for HR, found infrequently in Capsicum, are qualitative in their effect. They control highly useful resistance that is easily selected in breeding progenies. The genetic relation between two of the three reported genes for HR in pepper is unknown. The gene Bs_1 controls race-specific HR, but the degree of racial specialization, if any, of the genes Bs_2 and Bs_3 is unknown. Measurably different hypersensitive reactions may occur in pepper. Collectively these may control durable resistance. Durable resistance may also be controlled by simply inherited genes which do not mediate HR. This resistance is quantitative, and its inheritance may be basically additive. Poor understanding of the mode of inheritance of resistance may be resolved by assessing components of resistance in segregating progenies inoculated by infiltration of leaves with a carefully standardized low concentration of bacteria.

CHAPTER 3
THREE INDEPENDENT GENES FOR RESISTANCE TO BACTERIAL LEAFSPOT IN A PLANT INTRODUCTION OF PEPPER (*Capsicum annuum* L.)

Bacterial leaf spot, incited by *Xanthomonas campestris* pv. *vesicatoria* (Doidge, 1920), Dye 1978 (herein designated as Xcv) is the most destructive foliar disease of bell peppers in warm humid environments. Three strains of Xcv have been differentiated by their reaction after inoculation of pepper and tomato (*Lycopersicon esculentum* Mill.) plants. Isolates of the tomato strain are avirulent on pepper which reacts with hypersensitivity (Cook and Stall, 1969; Cook, 1973). Isolates virulent on pepper are divided into two strains, namely one to which tomato reacts with hypersensitivity, and the pepper strain which is virulent on both plant species (Cook and Stall, 1969; 1982). The pepper strain occurs world wide (Cook and Stall, 1982), while that which is avirulent on tomato has been reported from Brazil (Kimura et al., 1972).

Two races of the pepper strain were differentiated by inoculation of pepper plants carrying the resistance gene Bs_1. Race 2 induces a hypersensitive reaction (HR) in plants with Bs_1 but race 1 does not (Cook and Stall, 1969). The gene Bs_1 was first discovered in plants of pepper plant introduction PI 163192 (Cook and Stall, 1963). Releases of bell pepper Florida VR-2, Florida VR-4, and Delray Bell have Bs_1 (A.A. Cook, 1979, and 1982, personal communications). However, these are susceptible to race 1 so that gene Bs_1 alone is insufficient for disease control (Cook and Guevara, 1984; Cook and Stall, 1982; Dahlbeck et al., 1979).

Plants of PI 271322 were more resistant than others in tests by Sowell and Dempsey (1977). Resistance proved durable in diverse environments (Hibberd et al., 1979; Kim and Hartmann, 1985; Stall, 1981). Two inherited resistances to race 1 were identified in PI 271322. The line was heterogeneous for gene $\underline{Bs_3}$ which controlled hypersensitivity (Kim and Hartmann, 1985). Stall (1981) reported recessively inherited resistance controlled by one or two genes. This resistance was atypically hypersensitive and occurred in plants of PI 271322 lacking $\underline{Bs_3}$.

The difference between these resistances was observed only when leaves were infiltrated with appropriate concentrations of bacteria (Kim and Hartmann, 1985; Klement et al., 1964; Stall, 1981). Confluent necrosis controlled by $\underline{Bs_3}$ occurs within 24 h with inoculum density of 10^8 cfu (colony forming units) ml^{-1}. Necrosis occurs after 2 to 3 days in resistant plants which lack $\underline{Bs_3}$ (Cook and Stall, 1969; Kim and Hartmann, 1985). Recessively inherited resistance in segregating progenies derived from PI 271322 was assessed as fewer discreet lesions per unit area of leaf 2 to 3 weeks after inoculating with low concentrations of approximately 2.5×10^3 cfu ml^{-1} (Stall, 1981). Lesions also were smaller in resistant than in susceptible plants. Occasional flecks of small size may develop in plants with genes for HR when challenged with low inoculum concentration (Essenberg et al., 1979; Klement et al., 1964; Turner and Novacky, 1974).

Race 2 of the pepper strain of Xcv is the more common race in Florida and occurs with race 1 (Cook and Stall, 1982). Sowell and

Dempsey (1977) originally reported resistance to race 2 in PI 271322. I observed HR to race 2 in PI 271322 in addition to previously described responses, and postulated that three resistances were functional within the line, and all contribute to durable resistance. This chapter presents evidence for independent inheritance of three resistance mechanisms in PI 271322, and genetic evidence for a new race 3 of the pepper strain of Xcv.

Materials and Methods

Inoculum Preparation

The Xcv isolates used in these studies were stored frozen in 15% glycerol or in refrigerated, sterilized water. Inocula were prepared from agitated (24 h) nutrient broth cultures. After centrifugation, bacterial pellets were resuspended in sterile tap water, and standardized colorimetrically to 50% light transmittance to approximate a density of 5×10^8 cfu ml^{-1}. These suspensions were either used directly for observing HR, or, for other experiments, were serially diluted to final concentrations of 1×10^3 to 3×10^3 cfu ml^{-1}, confirmed by replicated colony counts from 0.05 ml subsamples spread on nutrient agar plates. Inoculation with each race was by hypodermic infiltration of intercostal leaf tissues. Confirmation of race was by reaction of pepper lines near isogenic for $\underline{Bs}_1$ after infiltrating them with 5×10^8 cfu ml^{-1} inocula (Cook and Stall, 1969).

Heterogeneity of Resistance in PI 271322

An estimate of the variability in resistance of PI 271322 to two races of Xcv was obtained from a small population. Plants of this line, the susceptible control Early Calwonder (ECW), and its near isogenic

line 10 R with $\underline{Bs_1}$ gene (Dahlbeck et al., 1979), were raised in steamed peat-vermiculite mix in 10-cm plastic pots arranged in a greenhouse (temperature range 20 to 35 C). Rows of 8 plants were randomized. Plants were watered as required and treated four times during the experiment with approximately 0.4 g per pot of soluble 20:20:20 fertilizer.

Four fully expanded leaves per plant of PI 271322 and ECW were each inoculated with approximately 2.5×10^3 cfu ml^{-1} of each of four isolates Xv 0623, Xv 77-3A, and Xv 82-8 (all race 1), and Xv 82-7 (race 2). Each leaf was inoculated with all four isolates. A spot about 2 cm diameter was infiltrated with inoculum.

A single leaf was sampled from each plant at 11, 14, 21 and 32 days following inoculation. The numbers of lesions per 2 cm^2 of leaf within a perimeter imprinted by a cork-borer were counted at each inoculation site viewed under a dissecting microscope (magnified 2.5 X). The diameters of five randomly chosen lesions at each site, or of all lesions where fewer than five existed, were measured at day 32 using a graduated eyepiece. In addition to this test, all plants of PI 271322, ECW, and 10 R were observed for development of HR at 24 h after inoculating two additional leaves per plant with 5×10^8 cfu ml^{-1} of each isolate. A single plant, designated 271-4, was selected for hypersensitivity to both races 1 and 2, as well as for a high degree of resistance to all isolates. The progeny of this plant was used in studies of inheritance of resistances.

Hypersensitivity to a total of 16 isolates belonging to races 1 and 2 was observed in a subsequent 166 plant population of PI 271322.

All plants in this test were inoculated with 5 x 10^8 cfu ml^{-1} inocula and reactions were compared with those of inbred progeny of 271-4.

Bacterial Populations in vivo

Low disease severity in PI 271322 was expected to correlate with lower populations of bacteria in mesophyll. Plants of 271-4, ECW, and 10 R were raised in a greenhouse as described above. Three fully expanded leaves per plant were each inoculated with three isolates each at approximately 1.5 x 10^8 cfu ml^{-1}: Xv 80-5 (race 1), Xv E3 (race 2), and Xv 69-1 which does not induce typical HR in 271-4. Single plants of each host were sampled at 0, 2, 5, 8, 11, and 14 days after inoculation. Populations of bacteria of each race were determined from replicated 1.0 cm^2 (i.e., 2 x 0.5 cm^2) leaf samples. Samples were triturated in 0.5 ml sterile water, the suspensions serially diluted where appropriate, and 0.05 ml subsamples of the final dilutions spread on nutrient agar plates. Colonies were counted after 2 to 3 days incubation at 30C, and mean values converted to $\log_{10}$ (cfu cm^{-2}) of leaf. The numbers of lesions per 2 cm^2 of leaf and diameters of a maximum of 5 lesions per inoculation site were obtained on additional plants 15 days after inoculation. The experiment was repeated.

Inheritance of Resistances

A single inbred progeny plant of 271-4 was crossed with single plants of cultivars Delray Bell (with the $\underline{Bs_1}$ gene) and ECW. Single F_1 plants of both crosses were self-pollinated to yield F_2 seed, and cross-pollinated with additional plants of respective parents to give back-cross progenies.

Several experiments were completed. The first was to test the allelism of $\underline{Bs_1}$ to HR of race 2 observed in 271-4, and to verify monogenic inheritance of $\underline{Bs_3}$. Population sizes were 12 plants of both parents Delray Bell and 271-4, 20 of F_1, 241 of F_2, 20 to 40 of the two backcrosses, and 5 each of control lines ECW and 10 R. Plants were grown in seedling flats in a greenhouse. The following inoculations were applied to the eight populations. When the cotylendonary leaves were fully expanded, one cotyledon on each plant was inoculated with isolate Xv 82-7 of race 2. Inoculum density was 5×10^8 cfu ml^{-1}. Hypersensitivity was observed 24 h after inoculation (Cook and Stall, 1969). On completion of that test, seedlings were transplanted to pots as described above. Isolates Xv 82-8 of race 1 and Xv 82-7 of race 2 were used to inoculate all seedlings at a more mature stage. A fully expanded leaf was infiltrated with inoculum at 5×10^8 cfu ml^{-1} and observed for hypersensitivity after 24 hours. The test was repeated on the same plants.

A second experiment comprised a series of plantings. The experimental goals were to investigate the inheritance of nonhypersensitive resistance in 271-4, to study any possible interaction of this resistance with genes for hypersensitivity to races 1 and 2, and to transfer resistance genes to a bell pepper background. Two separate plantings of the following seven populations were made in a greenhouse: parents 271-4 and ECW, F_1, F_2, backcrosses, and control line 10 R. Rows of 8 plants were randomized. Poor seed germination in the first planting prompted the second, and population sizes varied. Only mature leaves were inoculated. Isolates Xv 82-8 of race 1 and Xv 82-7 of race 2 were used to observe hypersensitivity as described above. All plants in

both plantings were inoculated with a low concentration (approximately 1.5×10^3 cfu ml^{-1}) of an isolate (Xv 77-3A) which does not induce typical HR in 271-4. Three fully expanded leaves adjacent the fork on the main stem were inoculated and harvested after 2.5 weeks. Lesions were counted and measured as described above, and mean values were obtained for each plant.

Genes for HR to races 1 and 2, whether in homozygous or heterozygous condition, were expected to result in few, small lesions in plants inoculated with low concentrations of bacteria (Essenberg et al., 1979; Turner and Novacky, 1974). All plants in the second planting were inoculated with approximately 1.5×10^3 cfu ml^{-1} of isolates Xv 82-8 (race 1) and Xv 82-7 (race 2). Lesions were counted and measured as described above, and mean values from three leaves per plant were obtained for each isolate.

Single plants of the first backcross to ECW were selected for all three resistance reactions. These were inbred and crossed with pollen from ECW plants. Resistant plants were selected in progenies of two successive cycles of recurrent backcrosses and inbred backcrosses. Population sizes varied. The same inoculation, evaluation, and selection procedures described above were used throughout. Cotyledon leaves also were inoculated in one set of progenies (i.e., third backcross). The following isolates were used in various tests: Xv 71-21, Xv 80-5, and Xv 82-8 of race 1, Xv 81-23, Xv 82-7, and Xv E3 of race 2, and Xv 69-1 of race 3.

Hypersensitivity to races 1 and 2 was expected to be qualitative (Cook and Stall, 1969; Kim and Hartmann, 1985). Nonhypersensitive

resistance is quantitative, and segregation may not result in discreet classes of plants (Adamson and Sowell, 1983; Hibberd and Gillespie, 1982a; Stall, 1981). In that event, data were evaluated by analyses of generation means weighted by their variances (Basford and De Lacy, 1979; Hayman, 1958; Mather and Jinks, 1971). This analysis requires no *a priori* assumption of the degree of dominance. The matrix parameter specification of Fisher (1918) was used, where *m* is the theoretical population mean at F_∞; *a* is the value of additivity, and *d* is value of dominance deviation. The analysis applies a X^2 goodness-of-fit test to computed parameters for a single-gene, additive-dominance model. In the event of poor fit, the analysis fits a digenic model with interactions (Basford and De Lacy, 1979). Specific comparisons of means were performed by t-test.

Results and Discussion

Heterogeneity of Resistances in PI 271322

All inoculated plants of PI 271322 were resistant to four isolates of Xcv comprising races 1 and 2. Only a few lesions per 2 cm^2 of leaf developed in leaves of PI 271322 infiltrated with approximately 2.5×10^3 cfu ml^{-1} (Table 3-1). This contrasted with relatively many lesions in ECW. Lesion diameter 32 days after inoculation was approximately 8 times as great in ECW as in PI 271322. Variation in lesion numbers but not in lesion diameter occurred among plants of PI 271322, and plants were identified with very few lesions with all four isolates. Plants of 10 R were uniformly hypersensitive within 24 h after inoculating with 5×10^8 cfu ml^{-1} of isolate Xv 82-7 of race 2. A susceptible reaction occurred in leaves of 10 R inoculated with race 1, and in leaves of ECW with both races. In these, watersoaked appearance of the

Table 3-1. Number of lesions per 2 cm^2 of leaf at timed intervals, and diameter per lesion at 32 days after inoculation of peppers Early Calwonder and PI 271322 with four isolates of Xanthomonas campestris pv. vesicatoria.

Xcv isolate Identity	Race	Host	Lesions per 2 cm^2 Days after inoculation[a] 11	14	20	32	Diameter per lesion at 32 days (mm)
Xv 0623	1	PI 271322	1.5 ± 0.32[b]	0.5 ± 0.22	0.3 ± 0.23	0.2 ± 0.14	0.1 ± 0
		ECW[c]	7.8 ± 2.13	15.7 ± 2.48	33.5 ± 4.15	41.5 ± 5.59	0.8 ± 0.04
Xv 77-3A	1	PI 271322	5.3 ± 1.31	6.7 ± 1.30	3.5 ± 0.95	4.9 ± 0.99	0.1 ± 0
		ECW	10.2 ± 3.40	19.6 ± 3.91	26.8 ± 5.04	52.8 ± 11.5	0.7 ± 0.06
Xv 82-8	1	PI 271322	6.0 ± 0.68	2.9 ± 0.96	1.7 ± 0.64	1.1 ± 0.40	0.1 ± 0
		ECW	8.2 ± 1.19	10.8 ± 2.24	18.6 ± 2.93	23.7 ± 4.08	0.7 ± 0.03
Xv 82-7	2	PI 271322	2.7 ± 0.93	3.6 ± 0.53	1.9 ± 0.65	1.8 ± 0.46	0.1 ± 0
		ECW	3.3 ± 1.28	6.7 ± 0.82	13.5 ± 2.69	18.0 ± 7.18	1.0 ± 0.05
General Mean		PI 271322	3.9	3.4	1.9	2.0	0.1
		ECW	7.4	13.2	23.1	34.0	0.8

[a]Inoculum concentration approximately 2.5 x 10^3 cfu ml^{-1}.
[b]Mean ± standard error of mean from 15 plants of PI 271322 and 6 plants of ECW.
[c]ECW = Early Calwonder

inoculated tissue occurred in 24 to 36 h after inoculation. This was followed by necrosis of the tissue in 2.5 to 3 days.

Heterogeneity for HR to both races 1 and 2 occurred among plants of PI 271322. Fourteen of 15 plants showed HR to isolates Xv 0623 and Xv 82-8 of race 1, four plants showed HR to isolate Xv 82-7 of race 2, and no plant was hypersensitive to isolate Xv 77-3A of race 1. One plant did not have HR to any isolate. The plants of PI 271322 and isolates of Xcv which together did not produce typical HR resulted instead in necrosis which differed also from the susceptible reaction in 10 R and ECW. This necrosis, which occurred in 2 to 3 days after inoculation, was dry and brown, and was not preceded by a watersoaked appearance. It will be designated as intermediate necrosis. Several single plants of PI 271322 were selected. One plant, designated 271-4, had HR with isolates Xv 0623, Xv 82-8, and Xv 82-7 of races 1 and 2 and very few lesions with isolate Xv 77-3A. Inbred progeny of 271-4 were homozygous for their reactions to these same isolates.

In a larger planting of PI 271322 (Table 3-2), four isolates of race 1, namely Xv 69-1, Xv 77-3A, Xv 81-18, and Xv 82-15 induced the intermediate necrosis in all plants of PI 271322 and four other isolates of race 1, namely Xv 0623, Xv 71-71, Xv 80-5, and Xv 82-8 induced typical HR in 123 (or 74.1%) plants. All 8 isolates of race 2, Xv 61-38, Xv 65-1, Xv 70-7, Xv 80-6, Xv 82-7, Xv 83-3, and Xv E3 induced HR in 65 (or 39.2%) plants. All other reactions occurring in PI 271322 were of the intermediate necrosis type. The frequencies of typical HR to isolates of races 1 and 2 were independent of each other (Table 3-2).

The HR to some isolates of race 1 was taken to represent the effect of gene Bs_3 (Kim and Hartmann, 1985). The race 1 isolates which

Table 3-2. Hypersensitivity in plants of PI 271322 to inoculation with four isolates of race 1 and eight isolates of race 2 of *Xanthomonas campestris* pv. *vesicatoria*.

Race 2 hypersensitivity[a]	Race 1 hypersensitivity[a]		Totals
	Present	Absent	
Present	47	18	65 (39.2%)[b]
Absent	76	25	101 (60.8%)
Totals	123 (74.1%)	43 (25.9%)	166

[a]Inoculum concentration approximately 5×10^8 cfu ml^{-1}.
[b]Percentage of total number of plants in parentheses.

did not induce typical confluent HR were classified temporarily as a new race 3. It was clear however that plants of PI 271322 were resistant to isolates of race 3. Plants which lacked HR to races 1 and 2 were nevertheless resistant to both of these races (Table 3-1). The HR reactions to races 1 and 2 therefore occurred in plants which already were resistant to all isolates. In fact, Stall (1981) used isolate Xv 80-5 to evaluate nonhypersensitive resistance in PI 271322. This isolate induced typical HR in plants with gene $\underline{Bs_3}$. The genetic relation of $\underline{Bs_1}$ (originally from PI 163192 and which controls HR to race 2) with the locus controlling HR to race 2 in PI 271322 is unknown.

Bacterial Population in vivo.

Populations of bacteria of races 1, 2, and 3 reached approximately 5×10^6 to 10^8 cfu cm^{-2} in leaves of ECW 10 to 14 days after infiltration with low inoculum concentration (Figure 3-1). In contrast, populations in leaves of 271-4, were 10^4 to 10^5 times lower than in leaves of ECW. Populations of isolate Xv E3 of race 2 were about 10 to 50 times higher in leaves of 10 R than in 271-4, and 10^3 to 10^4 times lower than in leaves of ECW. Changes in populations of races 1, 2, and 3 in leaves of 271-4 were consistent with hypersensitivity, that is, bacterial multiplication occurred for 2 to 3 days after which it ceased, and populations declined slowly (Cook and Guevara, 1984; Stall and Cook, 1968; Klement et al., 1964).

Many lesions of relatively large diameter developed in leaves of ECW inoculated with races 1, 2, and 3, and in 10 R leaves with races 1 and 3 (Table 3-3). Lesions were visible between 5 and 6 days after inoculation, corresponding to populations of 5×10^5 to 5×10^6 cfu per

Figure 3-1. Populations of bacteria per cm^2 of leaf of peppers Early Calwonder and 271-4 inoculated with races 1, 2, and 3, and pepper 10 R inoculated with race 2 of Xanthomonas campestris pv. vesicatoria. Points represent means of 6 replicates.

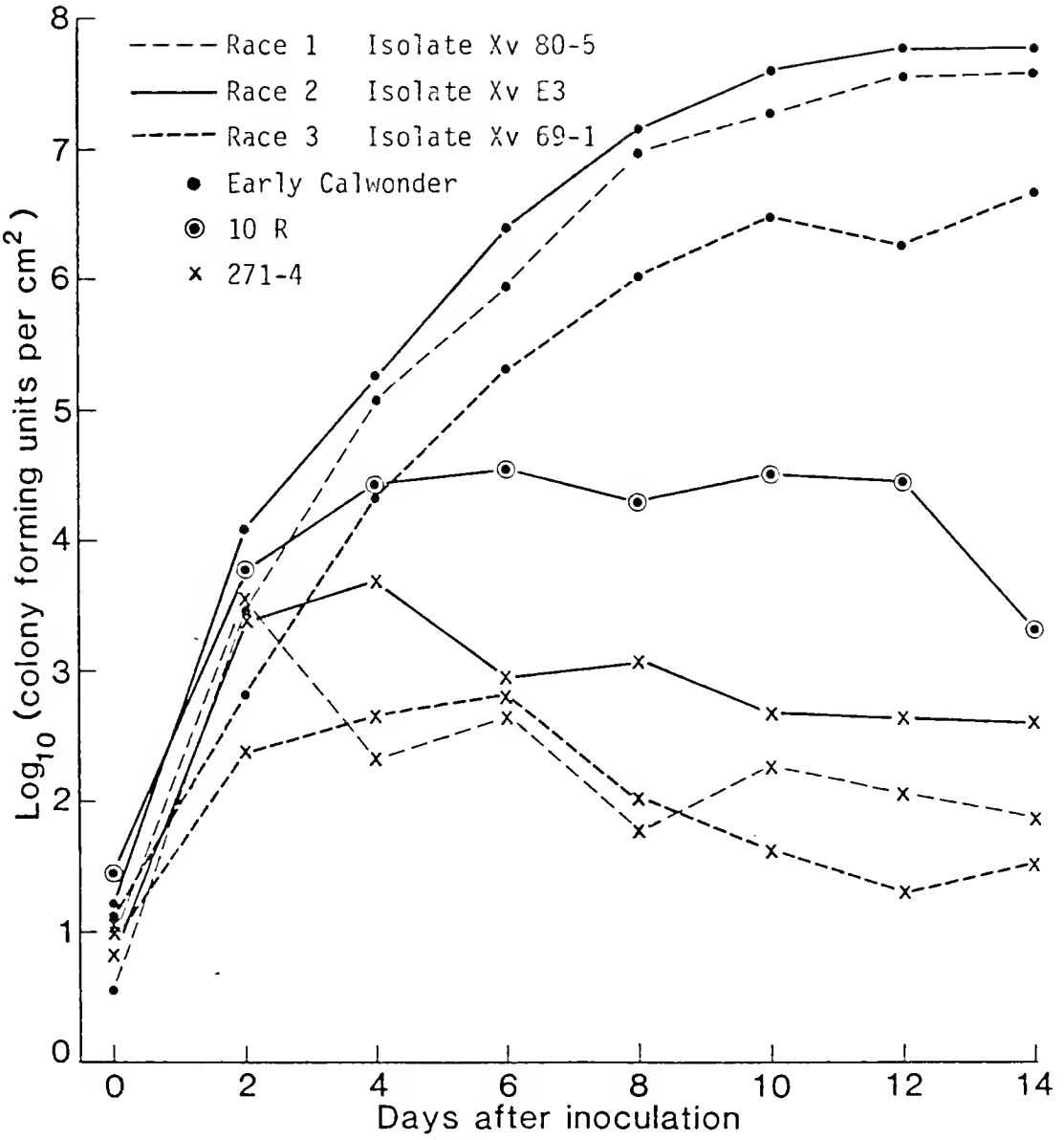

Race 1 Isolate Xv 80-5
Race 2 Isolate Xv E3
Race 3 Isolate Xv 69-1
Early Calwonder
10 R
271-4
Log10 (colony forming units per cm2)
Days after inoculation
0
1
2
3
4
5
6
7
8
0
2
4
6
8
10
12
14

Table 3-3. Number of lesions per 2 cm^2 of leaf and diameter per lesion in peppers 271-4, Early Calwonder, and 10 R 15 days after inoculation with races 1, 2, and 3 of Xanthomonas campestris pv. vesicatoria.

Xcv isolate[b]		Host					
		271-4		ECW[a]		10 R	
Designation	Race	Lesions per 2 cm^2	Diameter per lesion (mm x 10)	Lesions per 2 cm^2	Diameter per lesion (mm x 10)	Lesions per 2 cm^2	Diameter per lesion (mm x 10)
Xv 80-5	1	1.1	1.0	33.8	5.5	39.4	6.1
Xv E3	2	2.3	1.1	27.3	5.3	10.9	3.3[c]
Xv 69-1	3	0.2	1.0	8.6	2.9	11.6	3.4

[a]ECW = Early Calwonder
[b]Inoculum concentration of each isolate was approximately 1.5×10^3 cfu ml^{-1}.
[c]Necrotic hypersensitive flecks of irregular shape, unlike lesions with other combinations.

cm^2 (Figure 3-1). Only a few small lesions occurred in leaves of 271-4 with any isolate. Necrotic hypersensitive flecks developed in leaves of 10 R with isolate Xv E3 of race 2. Their higher frequency in 10 R than in 271-4 corresponded with bacterial populations approximately 10 times higher in 10 R (Figure 3-1).

Resistance to race 3 in 271-4 was clearly present and effective but was difficult to distinguish from hypersensitivity following inoculation with a low concentration of bacteria (Table 3-3, Figure 3-1). Interpretation of the mechanism underlying race 3 resistance should come from ultrastructural and physiological studies (Jones and Fett, 1985).

Inheritance of Resistance

Hypersensitivity to races 1 and 2

All plants of parents 271-4 and Delray Bell, and their F_1, F_2, and backcross progenies, and line 10 R, but none of ECW, were hypersensitive to isolate Xv 82-7 of race 2 (Table 3-4). This result occurred at two stages of plant maturity, namely in cotyledons of young seedlings and in fully expanded leaves of mature seedlings. This is consistent with the hypothesis that gene Bs_1 is present in homozygous condition in both parents 271-4 and Delray Bell.

All plants of 271-4, the F_1, and backcross (F_1 x 271-4) populations, but no plants of Delray Bell, 10 R, and ECW were hypersensitive to isolate Xv 82-8 of race 1 (Table 3-4). Segregation occurred in the F_2 and backcross (F_1 x Delray Bell) populations. Segregation was consistent with a ratio in the F_2 of 3:1 (X^2 = 1.33, P = 0.30 to 0.20), and a ratio in the backcross of 1:1 (X^2 = 0.71, P = 0.50 to 0.30). These ratios were expected if a single, homozygous gene, Bs_3, is in 271-4 (Kim and Hartmann, 1985).

Table 3-4. Segregation for hypersensitivity in progenies of peppers 271-4 and Delray Bell inoculated with races 1 and 2 of Xanthomonas campestris pv. vesicatoria.

	Numbers of plants[a]			
	Race 1 isolate Xv 82-8		Race 2 isolate Xv 82-7	
Generation	HR[b]	non-HR	HR	non-HR
271-4	12	0	12	0
Delray Bell	0	12	12	0
F_1 (271-4 x Delray Bell)	20	0	20	0
F_2	173	68	241	0
F_1 x 271-4	18	0	18	0
F_1 x Delray Bell	15	20	35	0
Controls				
Early Calwonder	0	5	0	5
10 R	0	5	5	0

[a]Inoculum concentration approximately 5×10^8 cfu ml^{-1}.
[b]HR = hypersensitive reaction.

Independent segregation of $\underline{Bs_1}$ and $\underline{Bs_3}$ occurred in progenies of 271-4 and ECW (Table 3-5). The F_1 progeny was hypersensitive to both races 1 and 2. Four combinations of HR to races 1 and 2 occurred in the F_2 populations (Table 3-5). In one planting, segregation was consistent with a ratio of 9 plants with HR to races 1 and 2:3 plants HR to race 1 only:3 plants HR to race 2 only:1 plant lacking HR. Segregation marginally failed to fit this ratio in the second planting (P = 0.05 to 0.02) (Table 3-5). Failure to fit resulted from unexpectedly few plants hypersensitive to isolate Xv 82-7 of race 2. Independent segregation of $\underline{Bs_1}$ and $\underline{Bs_3}$ was supported by data from backcross progenies in both plantings. All plants of the backcross (F_1 x 271-4) were hypersensitive to both races 1 and 2. Segregation in the backcross (F_1 x ECW) was consistent (P = 0.5 to 0.1) with the occurrence of equal frequencies of plants with each of the four possible combinations of hypersensitivity to races 1 and 2.

Single plants with both $\underline{Bs_1}$ and $\underline{Bs_3}$ were selected from the backcross (F_1 x ECW). These were both self-pollinated and backcrossed to ECW. Selection for $\underline{Bs_1}$ and $\underline{Bs_3}$ was subsequently made in progenies of two successive recurrent backcrosses to ECW (Tables 3-6 and 3-7). Observed segregation ratios adequately fitted those expected for independent assortment of both genes in all populations except one (Table 3-7). Significantly fewer plants hypersensitive to isolate Xv 81-23 of race 2 occurred in that population than expected. Similar deviations from expectation did not occur in any population inoculated with isolates of race 1. The results of inoculating leaves at two stages of plant maturity, namely recently fully expanded cotyledons and leaves of mature plants, agreed to within 98.5% (Table 3-7).

Table 3-5. Segregation for hypersensitivity in progenies of peppers 271-4 and Early Calwonder inoculated with races 1 and 2 of Xanthomonas campestris pv. vesicatoria.

Generation	Number of plants with each of the four possible genotypes[a]								Expected[b] ratio	X^2 value and P level
	Bs_1	Bs_3	bs_1	Bs_3	Bs_1	bs_3	bs_1	bs_3		
271-4	46[c]		0		0		0		-	-
	54		0		0		0		-	-
ECW[d]	0		0		0		49		-	-
	0		0		0		42		-	-
F_1 (271-4 x ECW)	51		0		0		0		1:0:0:0	-
	36		0		0		0			-
F_2	60		22		23		10		9:3:3:1	1.53 (P=0.5-0.2)
	138		54		35		23			7.62 (P=0.05-0.02)
F_1 x 271-4	60		0		0		0		1:0:0:0	-
	63		0		0		0			
F_1 x ECW	20		9		15		19		1:1:1:1	4.75 (P=0.2-0.1)
	29		25		25		18			2.59 (P=0.5-0.3)

[a]Race 1 isolate Xv 82-8 and race 2 isolate Xv 82-7 at inoculum concentration 5×10^8 cfu ml^{-1} were used to determine genotype.
[b]Ratio expected for independent segregation of two dominant genes, Bs_1 and Bs_3.
[c]Upper value from first planting; lower value from record planting.
[d]ECW = Early Calwonder.

Table 3-6. Segregation for hypersensitivity in progenies of the second backcross to Early Calwonder which, with controls were inoculated with races 1 and 2 of Xanthomonas campestris pv. vesicatoria.

Population	Number of plants with each of four possible genotypes[a]								Expected[b] ratio	X^2 value and P level
	Bs_1	Bs_3	bs_1	Bs_3	Bs_1	bs_3	bs_1	bs_3		
Controls										
271-4	29		0		0		0			
ECW[c]	0		0		0		15			
10 R	0		0		11		0			
Backcrosses										
(BC 11-2)[d] x ECW	41		39		28		32		1:1:1:1	3.17 (P=0.3-0.2)
(BC 31-1) x ECW	39		53		41		37		1:1:1:1	3.65 (P=0.2-0.1)
(BC 31-4) x ECW	15		12		17		14		1:1:1:1	0.90 (P=0.7-0.4)
Combined backcross total	95		104		86		83		1:1:1:1	2.94 (P=0.3-0.2)
(BC 31-4) selfed	62		20		14		4		9:3:3:1	2.68 (P=0.3-0.2)

[a]Race 1 isolate Xv 82-8, and race 2 isolate Xv 82-7 at inoculum concentration 5 x 10^8 cfu ml^{-1} used to determine genotype.
[b]Ratio expected for independent segregation of two independent dominant genes in female parents.
[c]ECW = Early Calwonder.
[d]Female parents refer to single plant selections from first backcross.

Table 3-7. Segregation for hypersensitivity in progenies of the third backcross which, with controls, were inoculated with races 1 and 2 of *Xanthomonas campestris* pv. *vesicatoria*.

Population	Number of plants with each of the four possible genotypes[a]								Expected[b] ratio	X^2 value and P level
	Bs_1	Bs_3	bs_1	Bs_3	Bs_1	bs_3	bs_1	bs_3		
Controls										
271-4	12		0		0		0			
ECW[c]	0		0		0		12			
10 R	0		0		4		0			
Backcrosses										
(BC 2-2-2)[d] x ECW	27[e]		48		37		38		1:1:1:1	5.89 (P=0.2-0.1)
	25		52		34		41			10.26 (P=0.02-0.01)
(BC 2-6-7) x ECW	47		34		38		32		1:1:1:1	3.52 (P=0.5-0.3)
	46		35		38		32			2.88 (P=0.5-0.3)
(BC 2-6-8) x ECW	43		39		36		37		1:1:1:1	0.74 (P=0.9-0.8)
	43		-[f]		-		-			
(BC 31-4-1 selfed) x ECW	139		0		0		0		1:0:0:0	--
	56[g]		-		-		-			

(BC 31-4-2 selfed) x ECW	72	80	0	0	1:1:0:0	0.42 (P=0.95-0.90)
	72	80	0	0		

[a]Race 1 isolate XV 71-21 and race 2 isolate Xv E3 at inoculation concentration 5 x 10^8 cfu ml^{-1} used to determine genotype.
[b]Ratios expected for independent segregation of two dominant genes in female parents.
[c]ECW = Early Calwonder.
[d]Female parents refer to single plant selections from second backcross.
[e]Upper value, numbers of seedlings in tests of cotyledonary leaves, lower value from tests of mature leaves.
[f]Mature plants not tested.
[g]All sampled plants were hypersensitive.

The genes $\underline{Bs_1}$ and $\underline{Bs_3}$ are inherited independently. Their phenotypes are dominant and observable in plants at two maturity stages. Use of cotyledon leaves to select $\underline{Bs_1}$ and $\underline{Bs_3}$ should contribute to efficient use of labor and time in backcross breeding. The low frequency of error in the cotyledon test with heterozygotes (Table 3-7) is not sufficient deterrent to its use. Selected plants may be reinoculated at a more mature stage.

Significantly fewer F_2 plants of the cross between 271-4 and ECW were hypersensitive to isolate Xv 82-7 of race 2 in one of two plantings (Table 3-5). In contrast, an excellent fit occurred in both plantings with isolate Xv 82-8 of race 1. It was possible that mutation of isolate Xv 82-7 occurred during inoculum preparation (Dahlbeck and Stall, 1979), and that plants in one test were consequently inoculated with a mixture of races. Two colony types in about equal frequency occurred in subsamples of inoculum which were cultured on nutrient agar plates. They proved to be races 2 and 3 by inoculating peppers 271-4, 10 R, and ECW (Chapter 4, this dissertation). Hypersensitivity to race 2 controlled by $\underline{Bs_1}$ predominates in plants inoculated with a mixture of races 1 and 2 (Stall et al., 1974). However, it is likely that HR developed slowly in this test in some plants with $\underline{Bs_1}$, and these were classified as nonhypersensitive (Table 3-5).

Inocula of isolate Xv 82-8 of race 1 and impure Xv 82-7, both at low concentration, were used to inoculate mature leaves of all plants. Low disease severity was expected in plants with $\underline{Bs_1}$ and $\underline{Bs_3}$ compared with those lacking these genes. Only a few lesions of small diameter occurred with isolate Xv 82-8 of race 1 in all plants of 271-4 and in its progeny which had $\underline{Bs_3}$ (Tables 3-8 and 3-9). In contrast, many

Table 3-8: Lesions per 2 cm^2 of leaf in peppers with four combinations of genes Bs_1 and Bs_3 following inoculation with races 1 and 2 of Xanthomonas campestris pv. vesicatoria.

Host population	Xcv[a] race	Lesions per 2 cm^2 of leaf in plants with each of the four possible genotypes							
		Bs_1	Bs_3	bs_1	Bs_3	Bs_1	bs_3	bs_1	bs_3
271-4	1	0.3 ± 0.08[b]		-		-		-	
	2	0.1 ± 0.05		-		-		-	
ECW[c]	1	-		-		-		29.0 ± 1.23	
	2	-		-		-		12.6 ± 0.71	
F_1 (271-4 x ECW)	1	0.6 ± 0.15		-		-		-	
	2	0.2 ± 0.07		-		-		-	
F_2	1	1.3 ± 0.13		1.1 ± 0.21		17.9 ± 2.27		14.2 ± 2.59	
	2	2.3 ± 0.13		4.9 ± 0.55		3.4 ± 0.55		6.0 ± 0.93	
F_1 x 271-4	1	0.8 ± 0.13		-		-		-	
	2	0.9 ± 0.16		-		-		-	
F_1 x ECW	1	1.9 ± 0.42		2.6 ± 0.45		27.4 ± 1.85		26.2 ± 1.86	
	2	5.5 ± 0.78		9.2 ± 1.43		3.8 ± 0.56		11.6 ± 1.35	

[a]Isolate Xv 82-8 of race 1 with inoculum concentration 2.0 x 10^3 cfu ml^{-1}; impure isolate Xv 82-7 of race 2 with concentration 1.2 x 10^3 cfu ml^{-1}.
[b]Mean ± standard error of mean.
[c]ECW = Early Calwonder.

Table 3-9: Diameters per lesion in peppers with four combinations of genes Bs_1 and Bs_3 following inoculation with races 1 and 2 of Xanthomonas campestris pv. vesicatoria.

Host population	Xcv[b] race	Diameter per lesion[a] (mm x 10) in plants with each of the four possible genotypes							
		Bs_1	Bs_3	bs_1	Bs_3	Bs_1	bs_3	bs_1	bs_3
271-4	1	1.0 ± 0[c]		-		-		-	
	2	0.1 ± 0.17		-		-		-	
ECW[d]	1	-		-		-		9.3	0.53
	2	-		-		-		9.5 ±	0.33
F_1 (271-4 x ECW)	1	1.0 ± 0.03		-		-		-	
	2	1.0 ± 0		-		-		-	
F_2	1	1.3 ± 0.08		1.4 ± 0.19		5.7 ± 0.53		4.3 ± 0.47	
	2	2.8 ± 0.19		5.8 ± 0.27		3.1 ± 0.33		6.0 ± 0.47	
F_1 x 271-4	1	1.1 ± 0.04		-		-		-	
	2	1.6 ± 0.13		-		-		-	
F_1 x ECW	1	1.7 ± 0.32		1.6 ± 0.38		7.0 ± 0.47		7.3 ± 0.41	
	2	3.8 ± 0.41		7.7 ± 0.26		2.6 ± 0.24		8.0 ± 0.49	

[a]Means of all non-zero values.
[b]Isolate Xv 82-8 of race 1 with inoculum concentration 2.0 x 10^3 cfu ml^{-1}; impure isolate Xv 82-7 of race 2 with concentration 1.2 x 10^3 cfu ml^{-1}.
[c]Mean ± standard error of mean.
[d]ECW = Early Calwonder.

lesions of large diameter occurred in ECW and backcross (F_1 x ECW) plants lacking $\underline{Bs_3}$.

Few lesions of small diameter with impure isolate Xv 82-7 of race 2 occurred in plants of 271-4, F_1 and backcross (F_1 x 271-4) progenies (Tables 3-8 and 3-9). In contrast, relatively many lesions of large diameter occurred in plants of ECW and in backcross (F_1 x ECW) plants lacking $\underline{Bs_1}$. Significantly fewer lesions of smaller diameter ($P<0.05$) occurred in F_2 and backcross (F_1 x ECW) plants with $\underline{Bs_1}$ than in sister plants lacking $\underline{Bs_1}$, but much overlap occurred between groups for both attributes. In addition, F_2 and backcross (F_1 x ECW) plants with $\underline{Bs_1}$ had significantly more and larger lesions ($P<0.05$) than plants of the parent 271-4, F_1, and backcross (F_1 x 271-4) populations. The F_2 and backcross (F_1 x ECW) plants with $\underline{Bs_1}$ appeared relatively far more diseased with impure isolate Xv 82-7 of race 2 than did sister plants with $\underline{Bs_3}$ inoculated with pure isolate Xv 82-8 of race 1.

Both $\underline{Bs_1}$ and $\underline{Bs_3}$ controlled dominant phenotypes with two widely different concentrations of inoculum. Mutation of race 2 to race 3 however confounded the degree of dominance with $\underline{Bs_1}$, and race 3 resistance varied independently of $\underline{Bs_1}$.

In a further test of the penetrance of $\underline{Bs_1}$ and $\underline{Bs_3}$, control lines and second backcross breeding progenies heterozygous for $\underline{Bs_1}$ and $\underline{Bs_3}$ were inoculated with low concentrations of isolates Xv 80-5 of race 1 and Xv E3 of race 2. Many lesions of relatively large diameter developed in leaves of ECW with both isolates, and in leaves of 10 R with isolate Xv 80-5 of race 1 (Table 3-10). In contrast, only a few lesions of small diameter developed in leaves of 10 R with isolate Xv E3, and with both isolates in 271-4 and hypersensitive backcross

Table 3-10. Lesions per 2 cm^2 of leaf and diameter per lesion in pepper control lines, and second backcross breeding progenies of genotype $\underline{Bs_1}$ $\underline{Bs_3}$ inoculated with races 1 and 2 of Xanthomonas campestris pv. vesicatoria.

Population	Number of plants	Race 1[a] Lesions per 2 cm^2	Race 1[a] Diameter per lesion (mm x 10)	Race 2[b] Lesions per 2 cm^2	Race 2[b] Diameter per lesion (mm x 10)
Controls					
271-4	7	1.6 ± 0.84[c]	1.1 ± 0.09	3.0 ± 2.20	1.1 ± 0.07
ECW[d]	8	103.4 ± 8.56	3.3 ± 0.21	21.9 ± 1.77	4.0 ± 0.39
10 R	4	102.8 ± 6.99	3.9 ± 0.30	10.3 ± 3.07	1.1 ± 0.06
Backcrosses					
(BC 11-2)[e] x ECW	40	12.9 ± 1.43	1.0 ± 0.02	11.2 ± 0.32	1.3 ± 0.04
(BC 31-1) x ECW	36	9.2 ± 1.01	1.0 ± 0.02	8.8 ± 0.74	1.1 ± 0.03
(BC 31-4) x ECW	13	14.0 ± 2.32	1.1 ± 0.06	11.7 ± 1.84	1.3 ± 0.10
(BC 31-4) selfed	61	5.2 ± 0.62	1.1 ± 0.03	10.5 ± 0.82	1.1 ± 0.02

[a]Isolate Xv 80-5 of race 1.
[b]Isolate Xv E3 of race 2.
[c]Mean ± standard error of mean.
[d]ECW = Early Calwonder
[e]Female parents refer to single plant selections from first backcross.

progenies. Significantly more lesions of both isolates occurred in backcross progenies heterozygous for $\underline{Bs_1}$ and $\underline{Bs_3}$ than in 271-4, but lesion diameters were equally small. The dominance of $\underline{Bs_1}$ and $\underline{Bs_3}$ was therefore slightly incomplete with low concentration inoculum.

Nonhypersensitive resistance to race 3

Plants of 271-4 were resistant to isolates of race 3 (Tables 3-1 and 3-2, Figure 3-1). Their reaction to inoculum containing 10^8 to 5×10^8 cfu ml^{-1} was distinguishable from the susceptible reaction by careful scrutiny, but the difference was not instantly recognizable (Cook and Stall, 1969). Segregates in F_2 progenies that were resistant to race 3 were not recognized with certainty, however, in preliminary tests with this inoculum density. It was necessary to quantify resistance in terms of two of its components, namely, number of lesions per 2 cm^2 of leaf (Stall, 1981) and diameter per lesion, following inoculation with low inoculum density. These components together reflected resistance to race 3 better than either alone (Figure 3-1, Table 3-3, and Chapter 8 of this dissertation).

The same experimental populations discussed above were inoculated with isolates of race 3 at low inoculum density. Large differences in means of both components occurred among the populations of parents 271-4 and ECW, F_1, F_2, backcrosses, and control line 10 R in two plantings inoculated with isolate XV 77-3A of race 3 (Table 3-11). Few lesions of small diameter occurred in plants of 271-4, F_1, and backcross (F_1 x 271-4) populations, and all plants appeared resistant (Tables 3-12, and 3-13). Relatively many lesions of large diameter occurred in plants of ECW and 10 R, but variation in lesion number in ECW was unexpectedly great in the second planting (Tables 3-11, 3-12 and 3-13). Wide variation in both components occurred among F_2 and backcross (F_1 x ECW) plants

Table 3-11. Lesions per 2 cm^2 of leaf and diameter per lesion in parents 271-4 and Early Calwonder, F_1, F_2, and backcrosses and control line 10 R in two plantings inoculated with race 3 of Xanthomonas campestris pv. vesicatoria.

Generation	Number of plants	Lesions[a] per 2 cm^2	Diameter per lesion (mm x 10)
271-4	50[b]	0.5 ± 0.17[c]	1.3 ± 0.14
	44	0.8 ± 0.20	1.2 ± 0.08
ECW[d]	42	14.3 ± 0.48	7.1 ± 0.55
	42	19.0 ± 0.92	4.8 ± 0.16
F_1 (271-4 x ECW)	51	2.2 ± 0.47	1.7 ± 0.14
	36	0.5 ± 0.11	1.3 ± 0.07
F_2	116	5.8 ± 0.54	2.2 ± 0.16
	236	4.6 ± 0.33	2.3 ± 0.07
F_1 x 271-4	60	1.8 ± 0.33	1.3 ± 0.07
	77	1.0 ± 0.22	1.3 ± 0.05
F_1 x ECW	22	12.0 ± 1.56	4.0 ± 0.60
	94	12.4 ± 0.85	3.3 ± 0.12
10 R	14	18.5 ± 0.75	5.3 ± 0.70
	8	15.1 ± 1.59	4.7 ± 0.65

[a]Isolate Xv 77-3A of race 3 with inoculum concentration in first plantings of 1.1 x 10^3 cfu ml^{-1}, and 1.5 x 10^3 cfu ml^{-1} in second planting.
[b]Upper value from first planting, lower value from second.
[c]Mean ± standard error of mean.
[d]ECW = Early Calwonder.

Table 3-12. Frequency distribution of lesions per 2 cm^2 of leaf occurring in parents 271-4 and Early Calwonder, F_1, F_2, and backcrosses in two plantings inoculated with race 3 of Xanthomonas campestris pv. vesicatoria.

Generation	Lesions[a] per 2 cm^2 of leaf											
	0	>0 to <3	3 to <6	6 to <9	9 to <12	12 to <15	15 to <18	18 to <21	21 to <24	24 to <27	27 to <30	>30
	Number of plants											
ECW[b]					3[c]	18	13	6	1	1		
			2	2	2	3	8	6	11	6	2	
271-4	6	41	4									
	18	24	1	1								
F_1 (271-4 x ECW)	22	16	3	7	1							
	20	13										
F_2	15	38	18	11	13	9	8	3	1			
	35	85	48	22	23	11	8	4	1			
F_1 x ECW	0	3	2	3	0	8	2	1	1	2		
	1	18	8	10	9	10	11	11	8	6	1	1
F_1 x 271-4	20	26	9	3	2							
	22	49	4	0	2							

[a]Isolate Xv 77-3A
[b]ECW = Early Calwonder
[c]Upper number from first planting; lower number from second planting.

Table 3-13. Frequency distribution of diameter per lesion in parents 271-4 and Early Calwonder, F_1, F_2, and backcrosses in two plantings inoculated with race 3 of Xanthomonas campestris pv. vesicatoria.

Generation	Diameter per lesion[a] (mm x 10)								
	0	>0 to <2	2 to <4	4 to <6	6 to <8	8 to <10	10 to <12	12 to <14	>14
	Number of plants								
ECW[b]			10[c]	9	7	6	6	1	3
			8	29	5				
271-4	41	10							
	18	22	4						
F_1 (271-4 x ECW)	22	20	8	1					
	13	18	2						
F_2	15	52	42	3	2	1	1		
	35	76	120	6					
F_1 x ECW		4	11	3	1	2	1		
	1	11	54	26	2				
F_1 x 271-4	20	37	2						
	22	49	6						

[a]Isolate Xv 77-3A.
[b]ECW = Early Calwonder.
[c]Upper number from first planting; lower number from second.

in both plantings. Distinct, nonoverlapping classes of resistant and susceptible plants did not occur, and the F_2 distribution was strongly skewed toward resistance in both plantings (Tables 3-12, and 3-13). However, distribution of lesion numbers in the backcross (F_1 x ECW) populations suggested two classes of plants, one resistant or partly so, and one susceptible (Table 3-12). The pattern of variation among generations strongly implied simple genetic control with a high degree of dominance for low values of components of resistance.

Analysis of generation means weighted by their error variances were computed for both components. Data were first transformed by square root of (x + 0.5) to account for the large number of zero values in some populations. Single gene models marginally failed to fit the transformed means of both components from the first planting (Table 3-14). However, digenic models with additive epistasis fitted adequately Epistasis was negative for log lesion numbers and positive for log lesion diameter. Digenic models were necessary to fit data from the second planting. Estimated parameters however still reflected a high component of dominance for low numbers of lesions, and additivity for lesion diameter (Table 3-15). Unexpectedly wide variation in both attributes occurred in nonsegregating generations (Tables 3-12 and 3-13). This probably contributed to failure of data to fit single gene models.

A combination of additive and incompletely dominant gene action for components of resistance basically explained continuous variation in these populations. Dominance was incomplete for low lesion numbers. Stall (1981), however, reported dominance for <u>high</u> lesion numbers in related progenies. Additional data to resolve this contrast were

Table 3-14. Analysis of the transformed, weighted generation means of number of lesions per 2 cm^2 of leaf and diameter per lesion for one planting of progenies of peppers Early Calwonder and 271-4 inoculated with race 3 of Xanthomonas campestris pv. vesicatoria.

Generation	Transformed[a] Lesions per 2 cm^2 of leaf Mean	Variance of mean	Transformed[a] Diameters per lesion (mm x 10) Mean	Variance of mean
ECW[b]	4.00	0.0034	2.69	0.0093
271-4	0.88	0.0034	1.34	0.0020
F_1 (271-4 x ECW)	1.40	0.0148	1.45	0.0021
F_2	2.24	0.0121	1.59	0.0041
F_1 x ECW	3.35	0.0551	2.05	0.0148
F_1 x 271-4	1.32	0.0084	1.34	0.0007
Parameters[c]	Estimate	S. E. of estimate	Estimate	S. E. of estimate
m	3.14*	0.239	1.74*	0.085
a	1.57*	0.040	0.67*	0.045
d	-1.71*	0.325	-0.28	0.118
aa	-0.70*	0.244	0.28*	0.099
Goodness of fit test	$X^2_{2df} = 4.04 < X^2\ 0.95 = 5.99$		$X^2_{2df} = 0.33 < X^2\ 0.95 = 5.99$	

[a]Square root (x + 0.5) transformation.
[b]ECW = Early Calwonder.
[c]Parameters: m = theoretical mean at F∞; a = additivity; d = dominance deviation; aa = additive epistasis.
*Significantly different from 0 at P<0.05.

Table 3-15. Analysis of transformed, weighted generation means of number of lesions per 2 cm^2 of leaf and diameter per lesion for the second planting of progenies of peppers Early Calwonder and 271-4 inoculated with race 3 of *Xanthomonas campestris* pv. *vesicatoria*.

Generation	Transformed[a] Lesions per 2 cm^2 of leaf		Transformed Diameters per lesion (mm x 10)	
	Mean	Variance of mean	Mean	Variance of mean
ECW[b]	4.35	0.0126	2.29	0.0011
271-4	1.04	0.0042	1.29	0.0007
F_1 (271-4 x ECW)	0.96	0.0022	1.27	0.0006
F_2	2.01	0.0047	1.65	0.0004
F_1 x ECW	3.35	0.0169	1.93	0.0009
F_1 x 271-4	1.12	0.0034	1.32	0.0003
Parameters[c]	Estimate	S. E. of estimate	Estimate	S. E. of estimate
m	1.80*	0.401	2.05*	0.045
a	1.66*	0.065	0.50*	0.021
d	1.70	1.034	-0.77*	0.063
aa	0.90*	0.395	-0.25*	0.051
ad	1.15*	0.313	0.17*	0.075
dd	-2.53*	0.652	-	-
Goodness of fit test	Not applicable		X^2_{1df} = 2.63 <X^2 0.95 = 3.84	

[a]Square root (x + 0.5) transformation.
[b]ECW = Early Calwonder.
[c]Parameters: m = theoretical mean at F∞; a = additivity; d = dominance deviation; aa = additive epistasis; ad = additive x dominance epistasis; dd = dominance epistasis.
*Significantly different from 0 at P<0.05.

collected from second backcross progenies inoculated with isolate Xv 69-1 of race 3. Parental selections crossed with ECW to produce these populations were highly resistant to isolate Xv 77-3A of race 3, and possessed $\underline{Bs}_1$ and $\underline{Bs}_3$.

No lesions developed in plants of 271-4 in 2 weeks after inoculation. In contrast, many lesions of relatively large diameter developed in leaves of ECW and 10 R (Table 3-16). Mean values of both components in backcross progenies were lower than in control lines ECW and 10 R but wide variation occurred (Table 3-17). Mean values from the inbred backcross population were significantly lower than from the noninbred backcrosses and susceptible control lines ($P<0.05$), and wide variation also occurred (Tables 3-16 and 3-17). The numbers of lesions per 2 cm^2 of leaf separated basically into groups in both the noninbred and inbred backcross progenies (Table 3-17). Three groups of plants were evident in the inbred backcross. These were resistant plants with very few lesions, an intermediate group, and a susceptible group with many lesions. The group of highly resistant plants did not occur in noninbred backcross progenies. The number of lesions per 2 cm^2 and diameter per lesion were positively correlated ($r = 0.65$, $P<0.05$). These data are consistent with a single additive gene for resistance being expressed in this experiment. Additivity is reflected in intermediate disease severity in heterozygotes (Tables 3-16 and 3-17).

Both resistance and susceptibility to race 3 isolates occurred among plants with genes $\underline{Bs}_1$ and $\underline{Bs}_3$ (Table 3-16) or with any other combination of these genes (Tables 3-5, 3-12, and 3-13). Progenies with any combination of resistances could be recovered. Resistance to races 1, 2, and 3 are independent of each other.

Table 3-16. Number of lesions per 2 cm^2 of leaf and diameter per lesion in control lines and progenies of the second backcross to Early Calwonder inoculated with race 3 of *Xanthomonas campestris* pv. *vesicatoria*. All backcross progeny plants were of genotype Bs_1 Bs_3.

Generation	Number of plants	Lesions[a] per 2 cm^2	Diameter per lesion (mm x 10)
Controls			
271-4	7	0	-
ECW[c]	8	18.6 ± 3.15[b]	5.4 ± 0.64
10 R	3	20.3 ± 1.43	5.4 ± 1.00
Backcrosses			
(BC 11-2)[d] x ECW	40	13.5 ± 1.24	4.1 ± 0.28
(BC 31-1) x ECW	35	12.9 ± 1.22	3.8 ± 0.32
(BC 31-4) x ECW	13	12.2 ± 1.33	4.9 ± 0.58
(BC 31-4) selfed	60	7.5 ± 0.88	2.6 ± 0.17

[a]Isolate Xv 69-1 of race 3 used with inoculum concentration 1.3×10^3 cfu ml^{-1}.
[b]Mean ± standard error of mean.
[c]ECW = Early Calwonder.
[d]Female parents refer to single plant selections from first backcross.

Table 3-17. Frequency distribution of lesions per 2 cm^2 of leaf and diameter per lesion in pepper control lines and progenies of genotype $\underline{Bs_1}$ $\underline{Bs_3}$ from second backcross to Early Calwonder inoculated with race 3 of Xanthomonas campestris pv. vesicatoria.

	Lesions[a] per 2 cm^2 of leaf											Diameter per lesion (mm x 10)					
Generation	0	>0 to <3	3 to <6	6 to <9	9 to <12	12 to <15	15 to <18	18 to <21	21 to <24	24 to <27	27 to <30	0	>0 to <2	2 to <4	4 to <6	6 to <8	>8
	Number of plants																
ECW[b]					3	1			2		2			2	2	3	1
271-4	7											7					
Combined backcrosses	0	5	7	18	10	14	10	10	10	2	3	0	6	37	33	10	3
Inbred backcrosses	7	16	4	12	5	7	5	3	1	1		7	15	33	4	2	

[a]Isolate Xv 69-1; inoculum concentration 1.3 x 10^3 cfu ml^{-1}.
[b]ECW = Early Calwonder.

To illustrate additive and dominant gene action with race 3, data from two different experiments are plotted and compared with data from Stall (1981) in Figures 3-2 and 3-3. The theoretical frequency of an allele in F_1 and F_2 progenies is 0.5, and is 0.25 and 0.75 in respective backcross populations. Generation means of both components of resistance from 6 populations are plotted against these frequencies for isolate Xv 77-3A in Figure 3-2, and for four populations with isolate Xv 69-1 in Figure 3-3. In addition, the generation means of lesion numbers observed by Stall (1981) are plotted in Figure 3-3. Resistance to isolate Xv 77-3A was incompletely dominant, and generation means of both components were not strictly related linearly to the frequency of the allele from ECW (Figure 3-2, Tables 3-14 and 3-15). However, means with isolate Xv 69-1 were linearly related to allele frequency (Figure 3-3, Tables 3-16 and 3-17). In contrast, dominance occurred for high lesion numbers in data from Stall (1981), and generation means increased nonlinearly with frequency of the allele for susceptibility (Figure 3-3).

The three sets of experiments are not strictly comparable since different plants, growing conditions, and bacterial isolates were used. However, a general pattern emerged to explain inheritance of resistance to race 3. Dominance reversal of lesion numbers with nontypically hypersensitive resistance can be explained as a reduced rate of bacterial multiplication. The potential number of lesions is controlled by inoculum concentration (Essenberg et al., 1979; Stall et al., 1982; Turner and Novacky, 1974). Bacteria multiply <u>in situ</u> to populations controlled, in part, by the resistance mechanism functioning in the host (Chapter 4, this dissertation). A small increase in bacterial populations may be associated with a large increase in the number of lesions

Figure 3-2. Generation means of lesions per 2 cm^2 of leaf and diameter per lesion related to theoretical frequency of the allele for susceptibility in parents, F_1, F_2 and backcross pepper populations inoculated with race 3 of Xanthomonas campestris pv. vesicatoria. Data are from the first of two plantings.

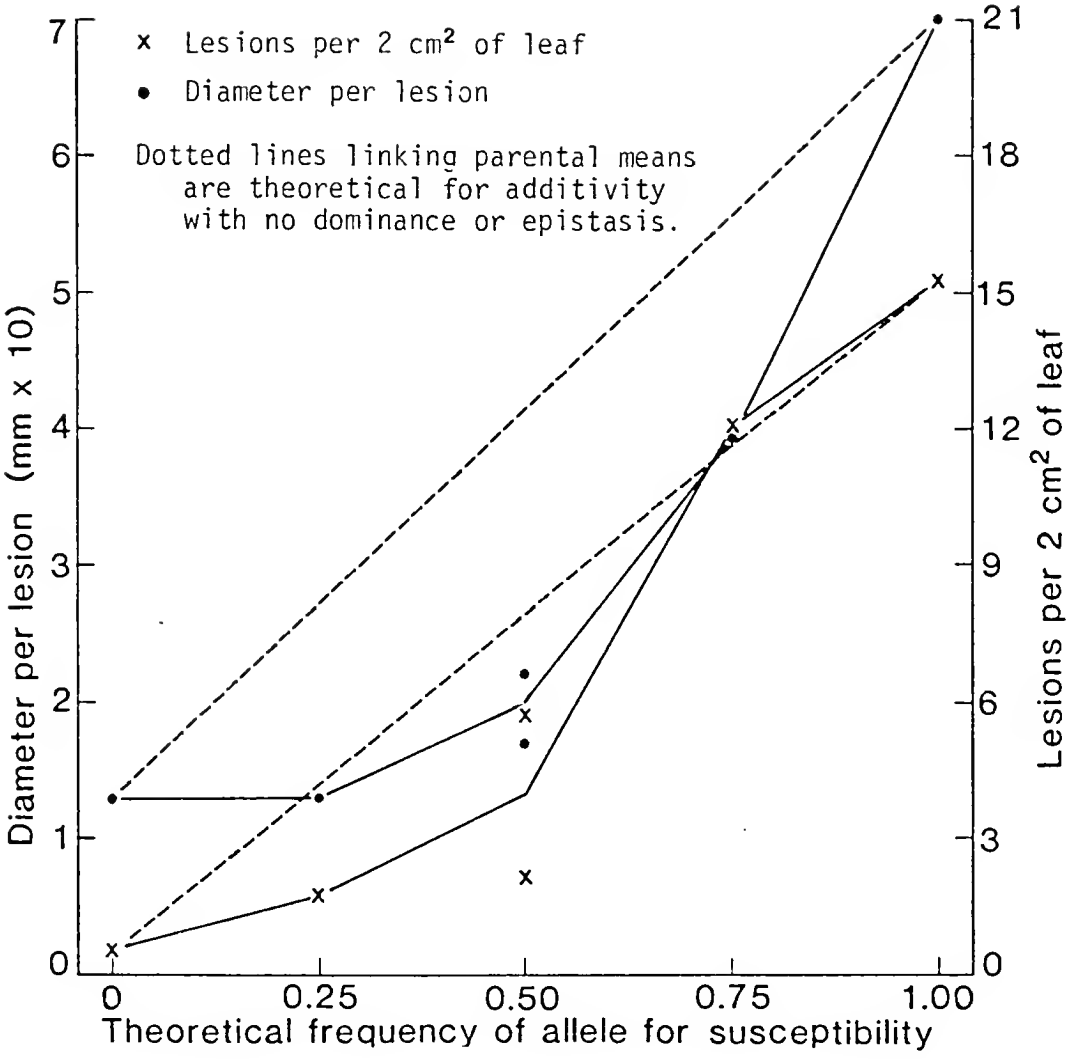
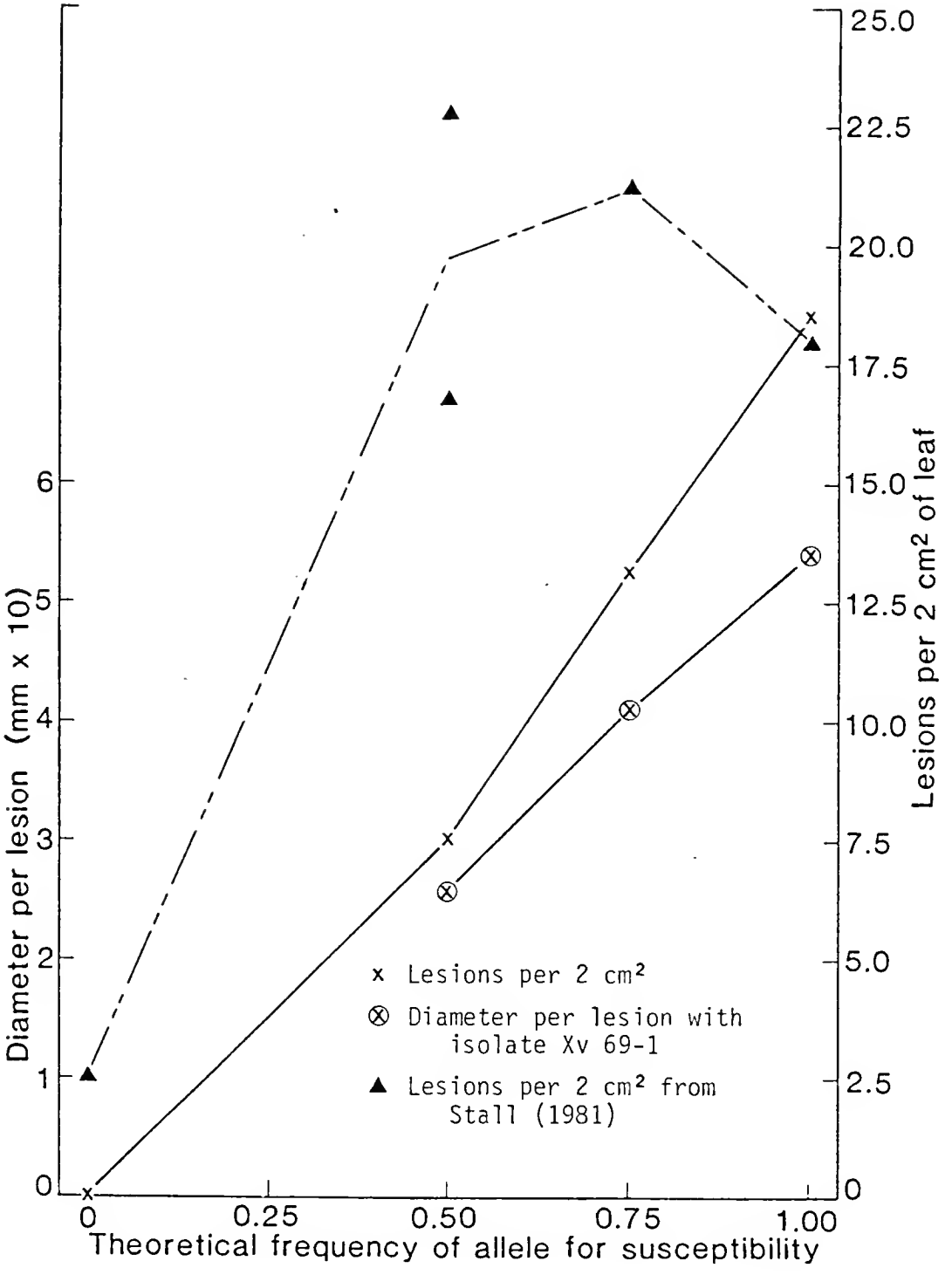

x Lesions per 2 cm² of leaf
• Diameter per lesion
Dotted lines linking parental means are theoretical for additivity with no dominance or epistasis.
Diameter per lesion (mm x 10)
Lesions per 2 cm² of leaf
Theoretical frequency of allele for susceptibility
0
0.25
0.50
0.75
1.00
0
1
2
3
4
5
6
7
0
3
6
9
12
15
18
21

Figure 3-3. Generation means of lesions per 2 cm^2 of leaf and diameter per lesion related to theoretical frequency of the allele for susceptibility in parents and second backcross breeding progenies inoculated with race 3 of Xanthomonas campestris pv. vesicatoria. Data of lesion numbers from Stall (1981) included for comparison.


Diameter per lesion (mm x 10)
Lesions per 2 cm² of leaf
Theoretical frequency of allele for susceptibility
0
0.25
0.50
0.75
1.00
0
1
2
3
4
5
6
0
2.5
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
25.0
x Lesions per 2 cm²
⊗ Diameter per lesion with isolate Xv 69-1
▲ Lesions per 2 cm² from Stall (1981)

that become visible (Chapter 8, this dissertation). Lesion expansion will continue whether few or all the potential lesions have become visible. The rate of lesion expansion will vary with the degree of resistance and aggressiveness of the isolate. Lesion diameter should approximate the mid-parent value characteristic of each isolate (see also Chapters 6 and 7 of this dissertation). It is clear that heterozygotes were identified in two successive recurrent backcrosses. These plants were identified by their components of resistance which reflect bacterial populations in host mesophyll (Figure 3-1, Table 3-3).

Reports of reversal of dominance and recessively inherited resistance to bacterial pathogens are common in the literature. For example Brinkerhoff et al. (1984), Chand and Walker (1964a and b), Fallik et al. (1984), Hibberd and Gillespie (1982a), Innes et al. (1982), Kim (1983), Patel (1982), Patel and Walker (1966), Sidhu and Khush (1978), Taylor et al. (1978), Valladares-Sanchez (1979), and Yoshimura (1984) have reported them. It is possible that underlying additivity may be occurring with many quantitatively-assessed resistance genes.

The PI 271322 is unique among investigated peppers in having three independent resistance mechanisms. All plants were resistant to all tested isolates of Xcv, but the line was heterogeneous for race-specific genes $\underline{Bs_1}$ and $\underline{Bs_3}$. In fact, both $\underline{Bs_1}$ and $\underline{Bs_3}$ were unnecessary for resistance to Xcv in PI 271322 and its progeny from crosses with bell pepper (Chapter 8, this dissertation; and R.E. Stall, 1982, personal communication). Resistance to race 3 therefore appeared to be generalized against all races. Nevertheless, all three genes contributed to make PI 271322 the most resistant observed by Sowell and Dempsey (1977).

These are useful either singly (Dahlbeck et al., 1979; Kim and Hartmann, 1985; Stall, 1981) or collectively. It should be noted, however, that plants with $\underline{Bs_1}$ and $\underline{Bs_3}$ may be highly susceptible to bacterial spot unless selected for resistance to race 3 (Table 3-17).

Efficient selection of resistance genes in segregating populations is vitally important in plant breeding. Genes $\underline{Bs_1}$ and $\underline{Bs_3}$ may be identified by infiltrating cotyledons with high concentration inocula. Single plants with resistance to race 3 were efficiently identified by inoculating fully expanded leaves with low concentration inoculum (Stall, 1981). The dominance reversal of race 3 resistance is of concern, and three approaches to selection are possible. Commonly, inbred backcross progenies are inoculated (Brinkerhoff et al., 1984; Hibberd and Gillespie, 1982; Innes et al., 1984; Patel, 1982; Stall, 1981), but two generations per cycle are required. Single generation cycles may be accomplished by progeny testing presumed heterozygotes (Hanson, 1959). Alternatively, heterozygotes may be identified by inoculating with a relatively weak growing isolate.

Clearly, dominant hypersensitivity is easier to select than nonhypersensitive resistance. Cook reported a single gene, $\underline{Bs_2}$, for hypersensitivity to race 1 of Xcv in a PI of C. chacoense L. (Cook and Guevara, 1982; 1984). The genetic relation between $\underline{Bs_2}$ and $\underline{Bs_3}$ and the reaction of race 3 on plants with $\underline{Bs_2}$ are the subject of a subsequent investigation (Chapter 5, this dissertation).

The designation $\underline{Bs_4}$ is proposed for the additively inherited, nonhypersensitive gene for resistance to race 3 of Xcv.

CHAPTER 4
DIFFERENTIATION OF PATHOTYPES OF *Xanthomonas campestris* pv. *vesicatoria* PATHOGENIC ON PEPPER (*Capsicum annuum* L.)

Pathogenicity on its host is the single most important characteristic of a pathogen. Races occur as variants which are virulent on plants with resistance genes. Two races of *Xanthomonas campestris* pv. *vesicatoria* (Doidge, 1920) Dye, 1978 (referred to here as Xcv) were recognized as pathogenic on pepper (*Capsicum annuum* L.) (Cook and Stall, 1969, 1982). Race 2 induced a typical rapid hypersensitive reaction (HR) in plants with gene Bs_1 (Cook and Stall, 1963; Stall and Cook, 1966), and race 1 did not. The gene Bs_1 was found first in plant introduction (PI) 163192 (Cook and Stall, 1963) and was recently confirmed to be also in PI lines 163189 and 271322 (this dissertation, Chapters 3 and 6).

Recently, Kim and Hartmann (1985) reported that PI 271322 has an independent gene, Bs_3, for HR to isolates of race 1. However, I observed variants of race 1 which did not induce typical HR in plants of PI 271322 with Bs_3 and presented genetic evidence for race 3 (this dissertation, Chapter 3). The PI 271322 was also resistant to race 3 but in an atypical hypersensitive way, and this resistance was inherited as a single additive gene Bs_4 in crosses with susceptible bell pepper. The three resistance genes in PI 271322 segregated independently (Stall, 1981; this dissertation, Chapter 3), and the line was resistant to Xcv irrespective of the presence or absence of Bs_1 and Bs_3.

The HR induced by race 2 in plants with Bs_1 was characterized by drastic host cell membrane disorganization and collapse within 24 h after inoculation (Stall and Cook, 1966). This change was reflected in rapidly increasing loss of electrolytes from the mesophyll inoculated with 10^8 cfu (colony forming units) ml^{-1} (Cook and Stall, 1968). Bacterial populations ceased to grow with these changes and sometimes progressively declined. Previously (Kim and Hartmann, 1985), no physiological evidence was presented to confirm the HR to race 1 in PI 271322, nor to distinguish between races 1 and 3 (this dissertation, Chapter 3). Changes in bacterial populations *in vivo* presented earlier (this dissertation, Chapter 3) were consistent with hypersensitivity to all races 1, 2, and 3.

The time from inoculation to observable hypersensitivity to fungal pathogens varies with the host gene for HR in several pathosystems (Day, 1974; Ellingboe, 1982; Keen, 1982). Evidence is accumulating for similar variations in time to cell collapse with HR to bacterial plant pathogens. Patel (1982) described two race-specific genes for HR with bacterial pustule in cowpea. The cell collapse with one race occurred later than cell collapse with the other race in the one plant with both resistances. Both Patel (1982) and Gitaitis (1983) described a third resistance to the same pathogen of cowpea. This response was characteristically more slowly developing than usually expected for HR, but was associated with low bacterial populations *in vivo*. Ersek and Hevesi (1983) and Long et al. (1985) noted slow developing hypersensitivity in soybean to bacterial blight. Cook (1973) characterized the slow developing HR in pepper to isolates of Xcv which are pathogenic only on

tomato. Electrolyte loss from inoculated leaf tissues was slower than with HR in the interaction between $\underline{Bs_1}$ and race 2, and populations of bacteria were correspondingly higher.

A definite sequence of events occurs in inoculated pepper plants with all three resistance genes ($\underline{Bs_1}$, $\underline{Bs_3}$ and $\underline{Bs_4}$) derived from PI 271322. Hypersensitivity occurs most rapidly with race 2 in 9 to 18 h, followed by HR with race 1 in 20 to 24 h, and lastly, atypical hypersensitive necrosis with race 3 in about 2 days after inoculating with 10^8 cfu ml^{-1}. In addition, HR in heterozygotes for $\underline{Bs_1}$ and $\underline{Bs_3}$ was delayed for 2 to several hours in comparison with homozygotes, although both genes control dominant phenotypes (Cook and Stall, 1963; Kim and Hartmann, 1985). Small necrotic lesions develop occasionally in hypersensitively resistant cotton (Essenberg et al., 1979), and pepper plants (this dissertation, Chapter 3) when challenged with low inoculum concentrations. Pepper plants heterozygous for $\underline{Bs_1}$ and $\underline{Bs_3}$ often developed more numerous but equally small lesions compared with homozygotes. This chapter presents evidence for races 1, 2, and 3 of Xcv, for differences in time to host reaction with the three resistance genes, and for incomplete dominance of genes $\underline{Bs_1}$ and $\underline{Bs_3}$ in pepper.

Materials and Methods

Inocula were prepared from agitated, late log phase, nutrient broth cultures. After centrifugation, pellets were resuspended in sterile tap water and standardized colorimetrically to 50% light transmittance to approximate a density of 5×10^8 cfu ml^{-1}. These suspensions were used either directly for observing HR and measuring electrolyte loss, or were serially diluted to 5×10^3 cfu ml^{-1} for determination of bacterial populations *in vivo*. Inoculation with

each race was by hypodermic infiltration of intercostal leaf tissues on each of three fully expanded leaves per plant. The following isolates were used as representative of Xcv: Xv 71-21 and Xv 80-5 of race 1, Xv E3 of race 2, and Xv 69-1 of race 3. They had been maintained frozen in 15% glycerol or on sealed nutrient agar plates at 3 to 4 C.

The single plant selection from PI 271322, designated 271-4, was used. Inbred progeny of 271-4 were homozygous for genes $\underline{Bs_1}$, $\underline{Bs_3}$, and $\underline{Bs_4}$ (this dissertation, Chapter 3). The susceptible cultivar Early Calwonder (ECW), and its near isogenic line 10 R with the $\underline{Bs_1}$ gene (Dahlbeck et al., 1979) were also used. Single plants of 271-4 and ECW were raised, self-pollinated, and reciprocally cross-pollinated. Plants of 10 R, and inbred and hybrid progeny of ECW and 271-4 were raised in a greenhouse (temperature range 18 to 35 C) in steamed peat-vermiculite mix in 10-cm plastic pots, and fertilized four times during the experiments with 0.4 g per pot of soluble 20:20:20 fertilizer.

Two repeated experiments were completed. The first was to determine differences in rates of electrolyte loss from leaves of peppers 271-4, ECW, and 10 R inoculated with representative cultures of races 1, 2, and 3. After inoculation, plants were maintained in constant temperature chambers at 25 ± 0.25 and 30 ± 0.25 C and illuminated for 18 h per day by fluorescent and incandescent lamps. The second experiment was to determine the degree of dominance of the three resistance genes in 271-4 and its hybrids with ECW by 3 methods. These methods were time after inoculation to visible plant reactions at 25 C, loss of electrolytes from inoculated leaf tissue of plants at 25 C, and bacterial populations $\underline{in\ vivo}$ in the same plants reinoculated after returning them to the greenhouse.

Electrolyte losses were determined with samples of 3.0 cm^2 of leaf tissue harvested at timed intervals after inoculation with 5 x 10^8 cfu ml^{-1}. Samples were suspended in 3.0 ml deionized water and conductivity in µmho of the suspending solution was recorded immediately. Conductivity was recorded again after vacuum infiltration at 63 cm Hg for 60 second and followed by agitation for 1 h at 30 C. The difference in conductivity between the two readings was taken to represent the influence of bacteria on host tissue (Cook and Stall, 1968). There were three replicates and the experiment was repeated.

Bacterial populations in vivo were determined from 1.0 cm^2 (i.e., 2 x 0.5 cm^2) samples of inoculated leaf tissue harvested at timed intervals after inoculation with 5 x 10^8 cfu ml^{-1}. Samples were triturated in 0.5 ml sterile tap water, the suspensions serially diluted where appropriate, and 0.05 ml of the final dilutions spread on nutrient agar plates. The resulting colonies were counted 2 to 3 days after incubation at 30 C, and numbers converted to $\log_{10}$ (cfu cm^{-2}) of leaf. Means of three replicates were recorded, and the experiment was repeated.

Results and Discussion

Electrical conductivity of the solutions containing discs from inoculated leaves increased slowly with all isolates in ECW, and with isolates Xv 80-5 (race 1) and Xv 69-1 (race 3) in 10 R (Tables 4-1 and 4-2; Figures 4-1 and 4-2). Greater increases occurred at 30 than 25 C. Averages of the means from two temperatures are shown in Figures 4-1 and 4-2. Visible watersoaking occurred at about 30 h accompanied by large increases in conductivity between 36 and 48 h.

Table 4-1. Conductivity as a measure of electrolyte loss at two temperatures from pepper leaf tissue inoculated with races 1, 2, and 3 of Xanthomonas campestris pv. vesicatoria.

Host variety	Xcv isolate	Electrical conductivity (μmho)[a] Hours after infiltration[b] 1	6	12	24	30	36	48
Early Calwonder	Xv 80-5	28[c]	43	48	110	149	150	351[d]
	race 1	39	26	47	63	91	123	198
	Xv E3	27	40	42	114	155	135	255[d]
	race 2	30	20	37	67	86	89	189
	Xv 69-1	30	42	48	112	145	129	261[d]
	race 3	38	21	41	67	85	107	192
10 R	Xv 80-5	37	48	65	82	87	92	-
		30	33	33	46	74	77	152[d]
	Xv E3	34	234	251	-	-	-	-
		34	159	298	-	-	-	-
	Xv 69-1	27	43	41	88	120	120	-
		41	17	38	46	85	118	176[d]
271-4	Xv 80- 5	65	61	84	274	338	-	-
		72	59	81	294	348	-	-
	Xv E3	68	193	263	-	-	-	-
		72	95	346	-	-	-	-
	Xv 69-1	66	58	77	103	121	125	225[d]
		65	85	60	144	148	245	173

[a]Values are means of 6 replicates except where indicated.
[b]Inoculum concentration 5×10^8 cfu ml^{-1}
[c]Upper value from 30 C treatment, lower value from 25 C.
[d]Means of 3 replicates.

Table 4-2. Conductivity as a measure of electrolyte loss from leaf tissue of parents Early Calwonder, 271-4, and their hybrids inoculated with races 1, 2, and 3 of Xanthomonas campestris pv. vesicatoria.

Host variety[c]	Xcv isolate	Electrical conductivity (μmho)[a] Hours after infiltration[b] 0	9	15	21	23	26.5	31	36
Early Calwonder	Xv 71-21 race 1	19	25	47	61	87	104	144	124
	Xv E3 race 2	24	34	60	85	107	127	185	190
	Xv 69-1 race 3	24	30	48	64	109	108	177	184
271-4	Xv 71-21	34	60	94	164	341	-	-	-
	Xv E3	39	347	-	-	-	-	-	-
	Xv 69-1	45	60	97	94	101	107	140	165
Reciprocal hybrids (pooled data)	Xv 71-21	26	42	76	127	194	221	-	-
	Xv E3	29	143	295	-	-	-	-	-
	Xv 69-1	29	38	69	73	115	107	125	141

[a]Values are means of 6 replicates for Early Calwonder and 271-4, and of 12 reps (pooled data) for hybrids between them.
[b]Inoculum concentration 5 x 10^8 cfu ml^{-1}
[c]Plants held at 25 ± 1 C

Figure 4-1. Conductivity as a measure of electrolyte loss from leaf tissue of peppers inoculated with races 1, 2, and 3 of Xanthomonas campestris pv. vesicatoria. Data were averaged for temperature treatments of 25 and 30 C, and points represent means of 12 replicates.

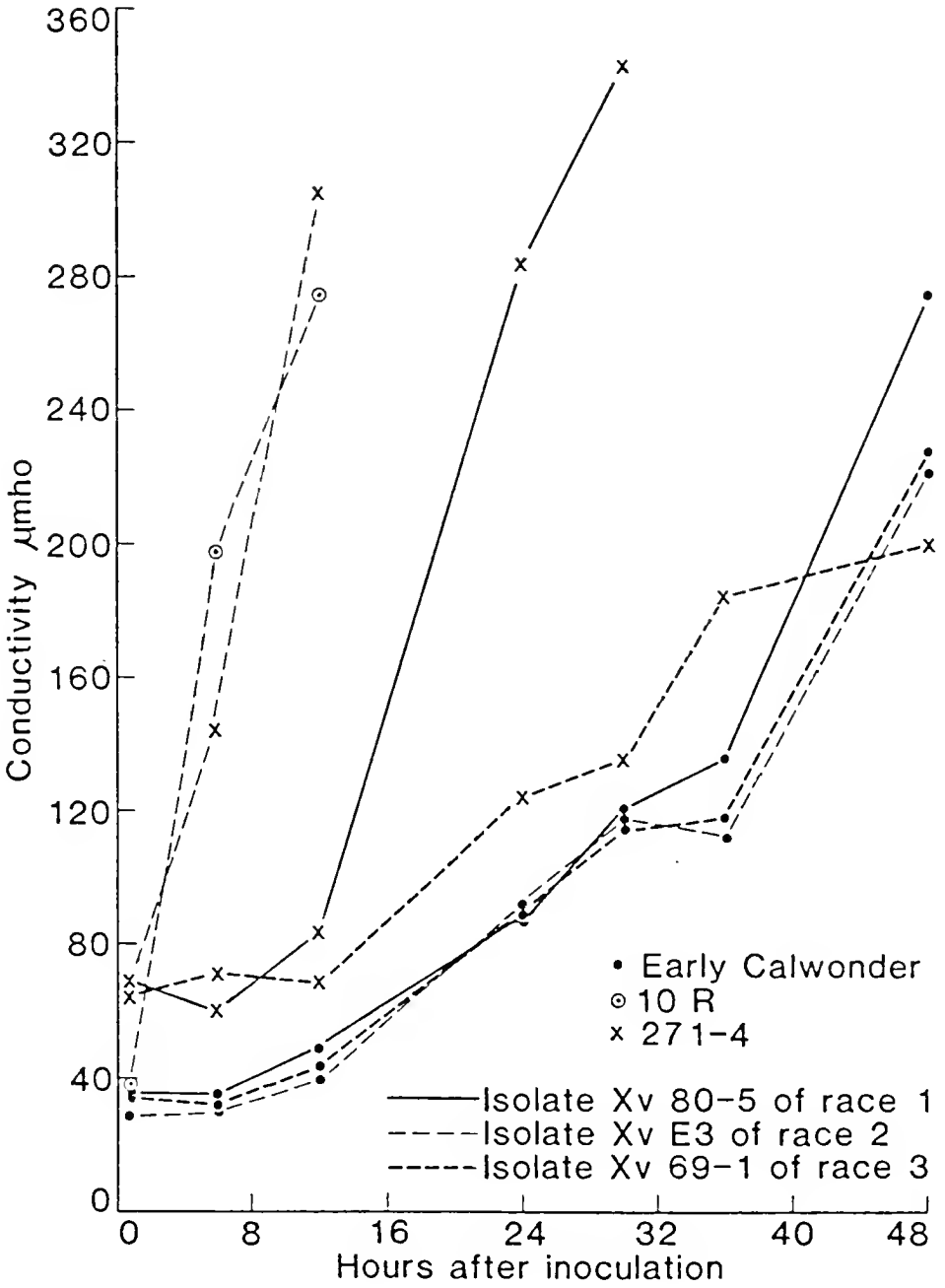
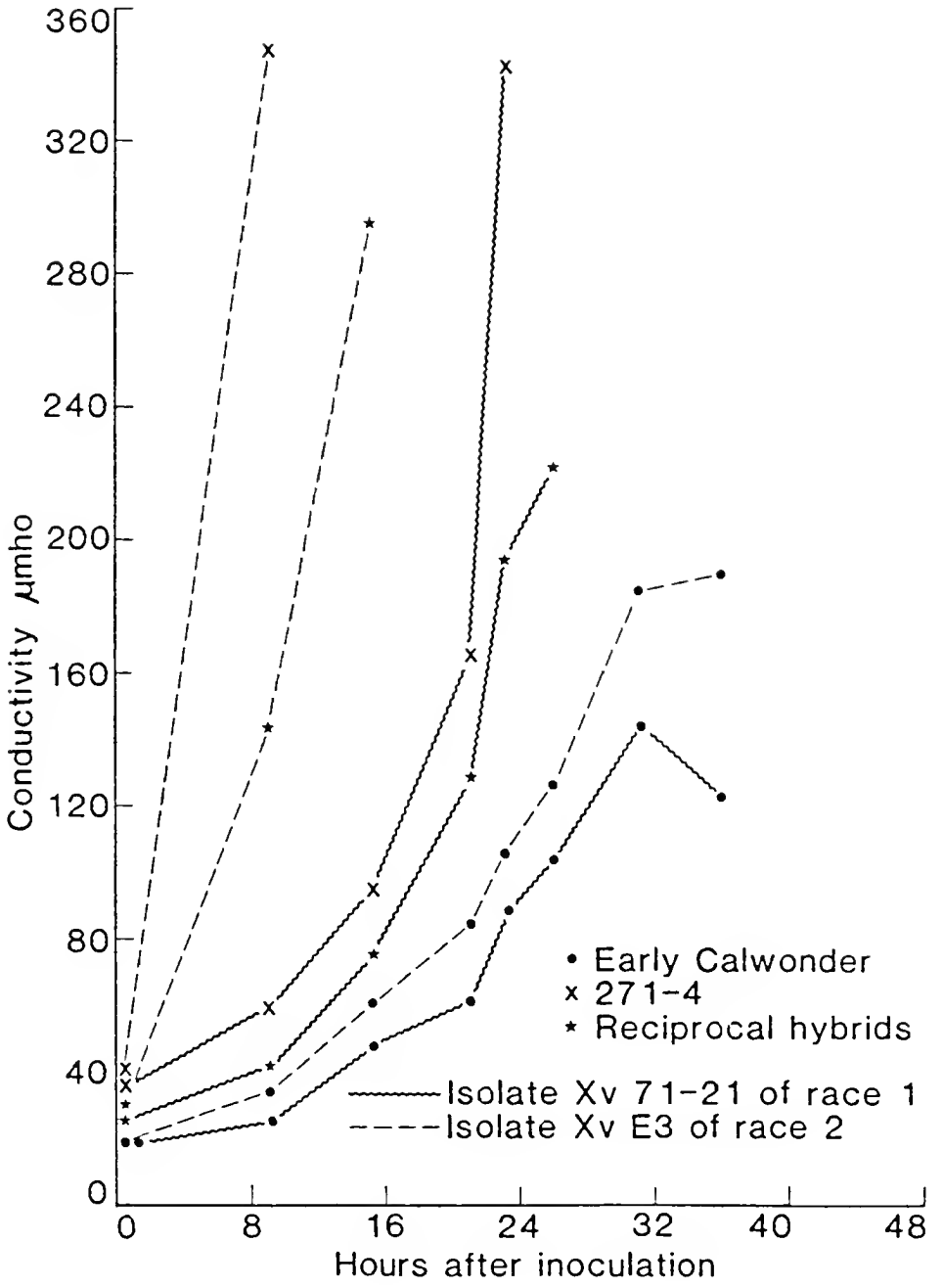

360
320
280
240
200
160
120
80
40
0
Conductivity µmho
0
8
16
24
32
40
48
Hours after inoculation
• Early Calwonder
⊙ 10 R
x 271-4
Isolate Xv 80-5 of race 1
Isolate Xv E3 of race 2
Isolate Xv 69-1 of race 3

Figure 4-2. Conductivity as a measure of electrolyte loss from leaf tissue of peppers Early Calwonder, 271-4 and their hybrids inoculated with races 1 and 2 of Xanthomonas campestris pv. vesicatoria. Points represent means of 6 or 12 replicates.


360
320
280
240
200
160
120
80
40
0
Conductivity µmho
0
8
16
24
32
40
48
Hours after inoculation
• Early Calwonder
x 271-4
★ Reciprocal hybrids
Isolate Xv 71-21 of race 1
Isolate Xv E3 of race 2

The lines 10 R and 271-4 were hypersensitive to isolate Xv E3 of race 2. The cell collapse appeared complete by 9 h after inoculation and was reflected by large increases in conductivity of the solution containing the leaf discs (Tables 4-1 and 4-2, and Figures 4-1 and 4-2). Slightly more rapid collapse occurred at 30 than 25 C (Table 4-1). Inoculated leaf tissue of both hybrids between 271-4 and ECW wilted after 9 h, and cell collapse was visibly complete by 13 to 14 h after inoculation (Tables 4-2 and 4-3, Figure 4-2). No difference occurred between the two hybrids and data from them were pooled.

The line 271-4, but not 10 R, was hypersensitive to isolates Xv 71-21 and Xv 80-5 of race 1 (Tables 4-1, 4-2, and 4-3). Wilting occurred in the inoculated tissue from about 15 h, and cell collapse was visibly complete within 24 h in all experiments. No marked difference in time to necrosis occurred between temperatures of 25 and 30 C. At 25 C, conductivity increased greatly between 21 and 23 h after inoculation (Table 4-2, Figure 4-2). Hypersensitivity was delayed several hours in hybrid plants. Wilting of the inoculated tissue occurred after 17 h in hybrids, and cell collapse was visibly complete by 27 h (Tables 4-2 and 4-3, Figure 4-2). No difference occurred between hybrids.

Typical HR with isolate Xv 69-1 of race 3 did not occur in plants of 271-4 in any experiment (Tables 4-1, 4-2, and 4-3, Figure 4-1). However, inoculated leaf tissue appeared wilted between 15 and 22 h after infiltration, in unison with race 1 isolates. After that time the difference between races became apparent. Most leaf tissue infiltrated with isolate Xv 69-1 of race 3 ceased to appear wilted and returned to apparent full turgidity by 23 h (Table 4-3). Approximately 10% of the

Table 4-3. Sequence of reactions of peppers Early Calwonder, 271-4, and their hybrid progeny inoculated with races 1, 2, and 3 of Xanthomonas campestris pv. vesicatoria.

Host variety	Xcv race[a]	Reaction at 25 C: Hours after inoculation with 5×10^8 cfu ml^{-1} (0 — 9 — 15 — 21 — 23 — 26.5)
271-4	1	No symptom — Wilting — HR[b]
	2	No symptom — Wilting at 8 h progressing rapidly to HR
	3	No symptom — Wilting — No wilt;HR flecks
Early Calwonder	1,2,3	No symptoms progressing to slight softening and water soaking after about 24 h
Hybrids	1	No symptom — Wilting — HR
	2	No symptom — Wilting — HR
	3	No symptom — Wilting — No wilt;no HR flecks

[a]Race 1 isolates Xv 80-5 and Xv 71-21; race 2 isolate Xv E3; race 3 isolate Xv 69-1.
[b]HR = hypersensitive reaction.

tissue appeared as randomly scattered hypersensitive flecks. This appearance did not alter subsequently. Conductivity of the solution containing leaf discs increased slowly with time (Tables 4-1 and 4-2, Figure 4-1), but watersoaked necrosis and a large increase in conductivity between 36 and 48 h, characteristics of the susceptible reaction, did not occur. A similar response, but which was delayed by several hours, occurred with both hybrids (Tables 4-2 and 4-3). However, no hypersensitive flecks occurred in hybrids.

Isolates of races 1 and 2 were clearly differentiated by inoculation of plants of 271-4 and 10 R. Races 1 and 3 in 10 R resulted in susceptible reactions. The HR in 271-4 to race 1 occurred about 14 h later than HR to race 2. Race 3 was clearly differentiated in plants of 271-4 by not inducing typical HR. Plants of ECW and 10 R were susceptible to race 3. The reaction in 271-4 to race 3 may be a modified or incomplete HR (Klement, 1982). Host cell metabolism appeared to be influenced by this reaction, and electrolyte loss occurred to an extent possibly sufficient to be associated with both restricted bacterial population increase (this dissertation, Chapter 3), and death of a small proportion of host tissue. The majority of host cells appeared to recover to a non-necrotic, turgid condition. This aspect needs verification by means of ultrastructural observations.

The phenotypes of Bs_1 and Bs_3 in heterozygotes were incompletely dominant following inoculation with high concentrations of bacteria. At 25 C the relative time delay in developing confluent HR necroses in heterozygotes compared with homozygous 271-4 was approximately 20% with Bs_1, and 50% with Bs_3. Hybrids were therefore expected to support

higher bacterial populations than in 271-4 (Klement et al., 1964), which did occur (Figures 4-3, 4-4, and 4-5).

Numerous discreet lesions with isolates of races 1, 2, and 3 were visible in leaves of ECW between 5 and 6 days after infiltration with low concentrations of inoculum. Lesions were not seen in 271-4. Occasional small lesions with isolate Xv E3 of race 2, and relatively many lesions with isolate Xv 69-1 of race 3 occurred in hybrids. Atmospheric conditions were at dew point during the nights of day 8 to 10. This encouraged natural watersoaking of the mesophyll by root pressure flow (Johnson, 1947). Watersoaking of leaf-tissue was noted at and adjacent to those inoculated sites where lesions were visible and bacterial populations were intermediate to high, but not where populations were low.

Populations of bacteria of races 1, 2, and 3 were 10^4 to 10^5 times lower in leaves of 271-4 than in ECW 10 to 14 days after inoculation (Figures 4-3, 4-4, and 4-5). No large or consistent differences occurred between the two hybrids, and data from them were pooled. Higher populations occurred in heterozygotes than in 271-4 but the degree of difference varied with the isolate. There was a small difference 4 to 10 days after inoculation with isolate Xv 71-21 of race 1 (Figure 4-3) but no difference remained after 12 to 14 days. The hybrid progeny had bacterial populations that were intermediate between the two parents with isolate Xv E3 of race 2 (Figure 4-4), and only 10-fold or less fewer than in ECW with isolate Xv 69-1 of race 3 (Figure 4-5).

The patterns of increase in populations of isolates Xv 71-21 and Xv E3 in 271-4 and heterozygotes were consistent with hypersensitivity controlled by $\underline{Bs_3}$ and $\underline{Bs_1}$, respectively (Cook and Guevara, 1984;

Figure 4-3. Populations of bacteria per cm^2 of leaf of peppers Early Calwonder, 271-4, and their reciprocal hybrids (pooled data) inoculated with race 1 isolate Xv 71-21 of Xanthomonas campestris pv. vesicatoria. Points represent means of 6 or 12 replicates.

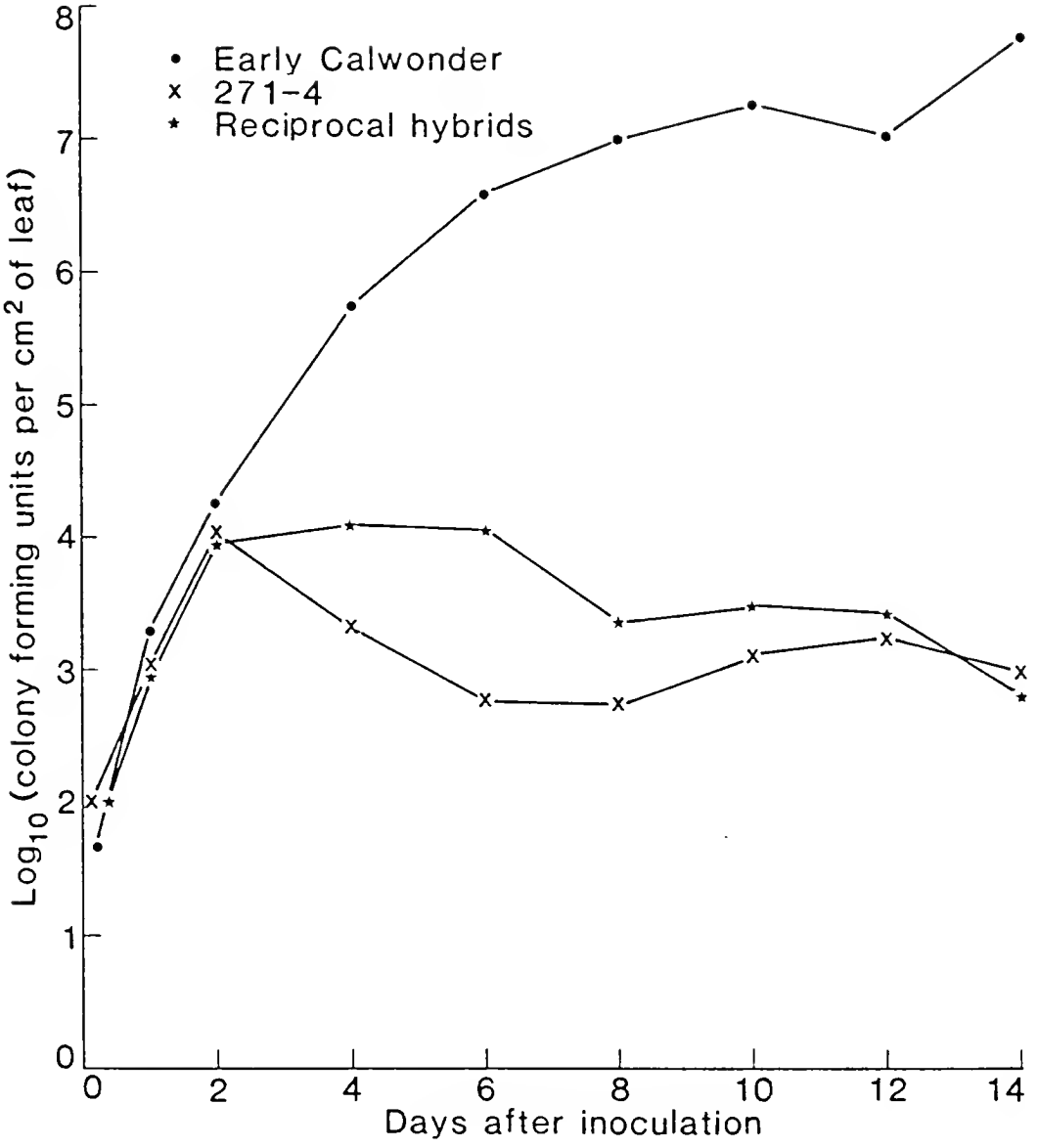

Early Calwonder
271-4
Reciprocal hybrids
Log10 (colony forming units per cm2 of leaf)
Days after inoculation
0
1
2
3
4
5
6
7
8
0
2
4
6
8
10
12
14

Figure 4-4. Populations of bacteria per cm^2 of leaf of peppers Early Calwonder, 271-4, and their reciprocal hybrids (pooled data) inoculated with race 2 isolate Xv E3 of Xanthomonas campestris pv. vesicatoria. Points represent means of 6 or 12 replicates.

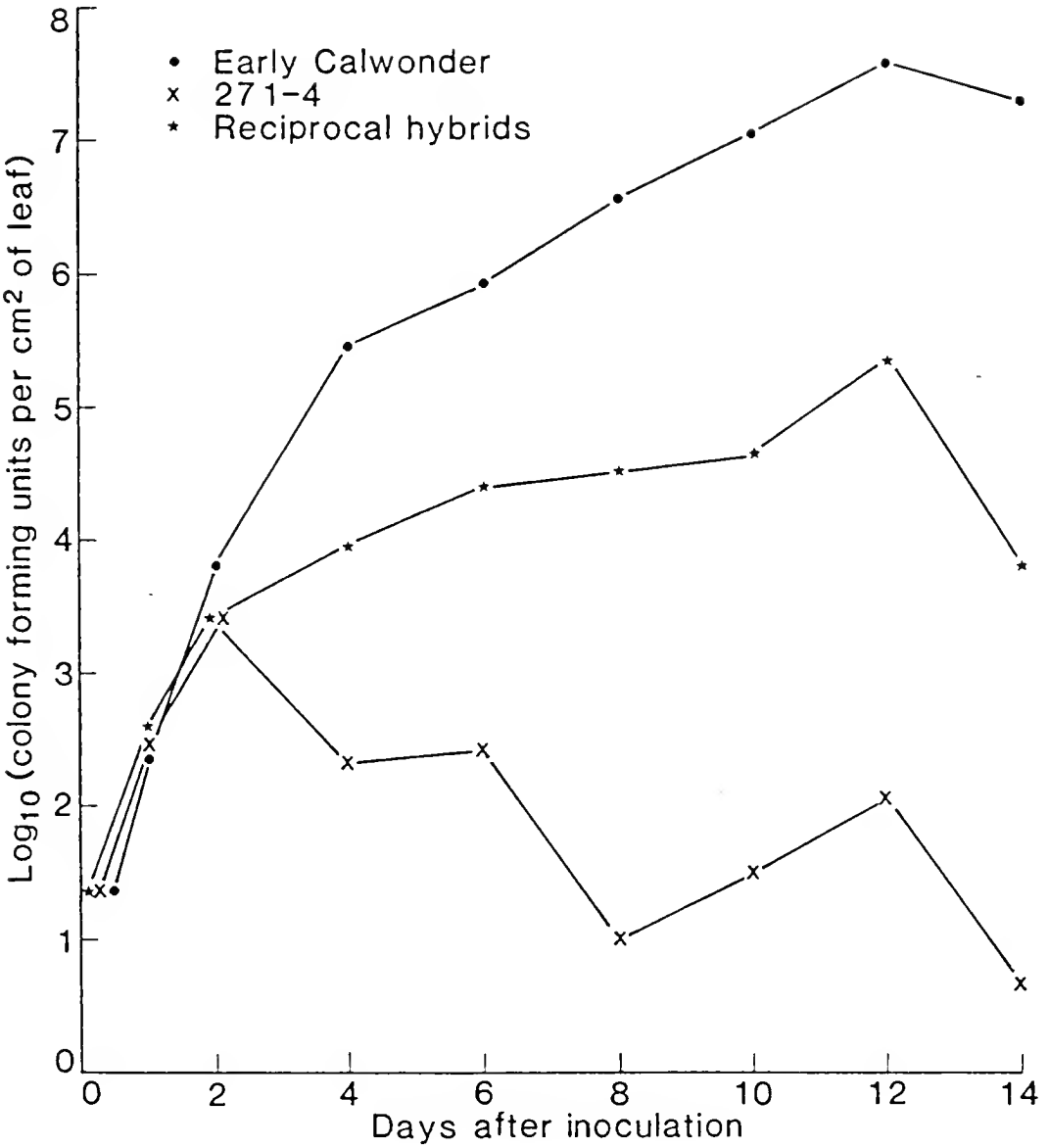
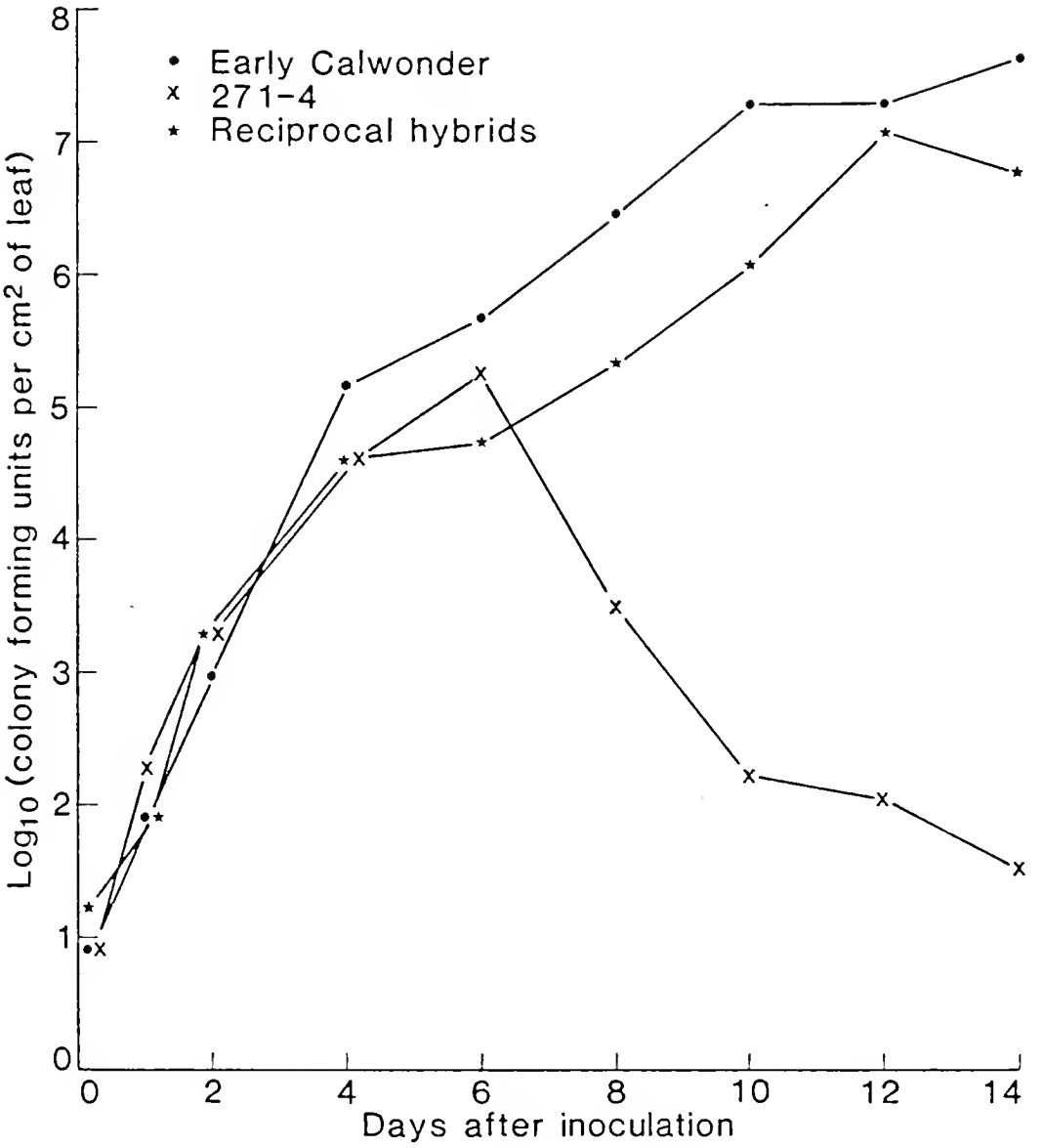

Early Calwonder
271-4
Reciprocal hybrids
Log10 (colony forming units per cm2 of leaf)
Days after inoculation
0
1
2
3
4
5
6
7
8
0
2
4
6
8
10
12
14

Figure 4-5. Populations of bacteria per cm^2 of leaf of peppers Early Calwonder, 271-4, and their reciprocal hybrids (pooled data) inoculated with race 3 isolate Xv 69-1 of Xanthomonas campestris pv. vesicatoria. Points represent means of 6 or 12 replicates.


Early Calwonder
271-4
Reciprocal hybrids
Log10 (colony forming units per cm2 of leaf)
Days after inoculation
0
1
2
3
4
5
6
7
8
0
2
4
6
8
10
12
14

Klement et al., 1964; Stall and Cook, 1966). Populations increased for approximately 2 days at rates similar to those in ECW leaves, but stabilized or declined slowly thereafter. Cell collapse within 27 h at 25 C in both homozygotes and heterozygotes followed infiltration of leaves with races 1 and 2 at high inoculum concentrations. Bacteria multiplied for two days in the variable greenhouse environment after which multiplication ceased with low inoculum concentrations. In contrast to these results, populations of isolate Xv 69-1 of race 3 in 271-4 and heterozygotes increased for 4 to 6 days after inoculation before resistance was noted. Populations decreased greatly in leaves of 271-4 after day 6 (Figure 4-5) but continued to increase in heterozygotes, which appeared susceptible.

Natural watersoaking probably contributed to relatively high populations in heterozygotes with isolates Xv E3 and Xv 69-1 of races 2 and 3, respectively (Klement, 1982). The HR to race 2 controlled by Bs_1 is inhibited and a susceptible reaction occurs where constant watersoaking is enforced (Stall and Cook, 1979; and personal observations). Hypersensitive flecks with race 2 occurred in some leaves in noninoculated tissue adjacent to the inoculated zone. This implied free movement and multiplication of bacteria in a continuum of water. It should be noted however that intermediate populations occurred from day 4 before watersoaking was observed. The population of isolate Xv E3 was about 10 times lower at day 14 than at days 10 as 12. This is consistent with populations stabilizing in the absence of watersoaking.

Resistance to race 3 in PI 271322 is variously inherited as a recessive (Stall, 1981), partially dominant, or purely additive trait

(this dissertation, Chapters 3 and 5). The differences occur in proportion to the vigor of the isolates used and the environmental conditions (see also this dissertation, Chapters 6 and 7). In all cases, the data could be interpreted as single gene inheritance. In this experiment, the F_1 hybrids were susceptible to isolate Xv 69-1, but bacterial populations were about 10 times lower than in ECW leaves.

The genes $\underline{Bs_1}$ and $\underline{Bs_3}$ were selected by their dominant phenotypes in segregating populations (Cook and Stall, 1963; 1969; Kim and Hartmann, 1985). Examined more closely, both genes in 271-4 were incompletely dominant. The genes are contrasted in several ways. A relatively greater degree of dominance occurred in these experiments with $\underline{Bs_3}$ and isolate Xv 71-21 of race 1 than with $\underline{Bs_1}$ and isolate Xv E3. The time to induction of cell collapse with $\underline{Bs_1}$ is 3 to 5 h (Meadows and Stall, 1981) but is unknown with $\underline{Bs_3}$. Completion of latency of HR appeared to require much longer with $\underline{Bs_3}$ than $\underline{Bs_1}$, but once induced, disruption to host cell metabolism in heterozygotes with $\underline{Bs_3}$ was more potent than with $\underline{Bs_1}$ in these experiments. These differences suggest two pathways for induction of HR and may need to be considered in models of host-parasite interactions.

Resistance to multiplication of race 3 in 271-4 and heterozygotes required considerably longer induction period than with races 1 and 2. Once resistance was induced, more rapid bacterial cell death in 271-4 occurred with race 3 than with races 1 and 2 (Figures 4-3, 4-4, and 4-5). This is consistent with toxic substances in the leaf playing a larger role in debilitating bacteria of race 3 than of races 1 and 2. These results imply that this resistance mechanism may be different from that controlled by $\underline{Bs_1}$ and $\underline{Bs_3}$.

These data confirm the validity of selecting genes $\underline{Bs_1}$ and $\underline{Bs_3}$ on phenotype in segregating backcross progenies (Cook and Stall, 1963; Kim and Hartmann, 1985), but highlight the need for inbred backcrosses to observe resistance to race 3 (Adamson and Sowell, 1983; Hibberd and Gillespie, 1982a; Stall, 1981; this dissertation, Chapter 3). They also clearly show that pepper plants with resistance genes $\underline{Bs_1}$ and $\underline{Bs_3}$ may yet be susceptible to Xcv (this dissertation, Chapter 3). The PI 271322 is resistant to all races of Xcv irrespective of $\underline{Bs_1}$ and $\underline{Bs_3}$ (Kim and Hartmann, 1985; this dissertation, Chapter 3). Nonhypersensitive resistance is common in pepper plant introductions (Cook and Stall, 1969; Hibberd and Gillespie, 1982a; Kim, 1983; this dissertation, Chapters 6, 7 and 8), and in other pathosystems (Brinkerhoff et al., 1984; Chand and Walker, 1964a and b; Fallik et al., 1984; Innes et al., 1984; Patel, 1982; Patel and Walker, 1966; Taylor et al., 1978; Yoshimura et al., 1984). However, more effort is required to identify resistant segregates.

CHAPTER 5
ALLELISM OF RACE-SPECIFIC GENES FOR HYPERSENSITIVE RESISTANCES TO *Xanthomonas campestris* pv. *vesicatoria* IN TWO LINES OF PEPPER (*Capsicum annuum* L.)

Bacterial leaf spot incited by strains of *Xanthomonas campestris* pv. *vesicatoria* (Doidge, 1920) Dye 1978 (hereafter designated as Xcv) is the most destructive foliar disease of pepper (*Capsicum annuum* L.). Three races of the pepper strain have been differentiated by inoculating plants carrying dominant genes for hypersensitivity. Those genes were originally derived from plant introductions (PI) 163192 and 271322. Race 1 induces a typical hypersensitive reaction (HR) in plants with the Bs_3 gene from PI 271322 (Kim and Hartmann, 1985). Race 2 induces HR in plants with the Bs_1 gene, from PI 163192. The PI 271322 also carries this gene (Chapter 3, this dissertation). Race 3 fails to induce typical HR in plants with any combination of the two genes (Chapter 3, this dissertation). The tomato strain of the organism is not pathogenic on pepper, i.e. pepper reacts with hypersensitivity to this strain (Cook and Stall, 1969).

The release of bell pepper, Florida VR-2, has the Bs_1 gene (A. A. Cook, 1979, personal communication). Cook reported a single dominant gene, Bs_2, for hypersensitivity to Xcv Race 1, which he found in PI 260435 of *C. chacoense* L. (Cook and Guevara, 1982; 1984). The recent release, Florida XVR 3-25 (herein designated XVR 3-25) (Cook, 1984), was produced by recurrent back-crossing to Florida VR-2 with selection for Bs_2. The best procedure to detect genes for HR is to infiltrate a

high inoculum concentration (10^8 cfu [colony forming units] ml^{-1}) which results in confluent necrosis of inoculated mesophyll within 24 h (Cook and Stall, 1969; Kim and Hartmann, 1985). It should be noted that occasional small necrotic hypersensitive flecks may develop in response to a low inoculum concentration (2×10^3 cfu ml^{-1}) with the same races (Essenberg et al., 1979; Klement et al., 1964; Turner and Novacky, 1974).

The PI 271322 contains another genetic resistance which segregates independently of the two HR genes, Bs_1 and Bs_3, and controls resistance to race 3 (Stall, 1981; Chapter 3, this dissertation). This resistance is characterized by very few, small, discreet lesions (compared with relatively many more, larger lesions in susceptible material) 2 to 3 weeks after infiltrating inoculum into mesophyll at low concentrations of 2×10^3 cfu ml^{-1}. High inoculum concentrations produce in 2 to 3 days a necrosis intermediate in appearance between typical HR and susceptible host reactions and similar to the description given by Cook and Stall (1969).

Evidence that Bs_1 (from XVR 3-25) and Bs_3 (from PI 271322) are different loci controlling HR to race 1 was lacking. In addition I observed that XVR 3-25 was hypersensitive to race 3 isolates and speculated that it may have an additional gene for HR not previously observed. This paper reports evidence to confirm hypersensitivity in XVR 3-25 to all three races, and the results of an investigation of allelism of resistance genes in XVR 3-25 and PI 271322.

Materials and Methods

Inoculum Preparation

The Xcv isolates Xv 80-5 (race 1), Xv E3 (race 2), and Xv 69-1 (race 3) were used throughout the studies of allelism of genes and

electrolyte losses from inoculated tissues. They had been stored frozen in 15% glycerol. Additional isolates used on plants of XVR 3-25 were race 1 isolates Xv 0245, Xv 0623, Xv 71-21, Xv 77-3, race 2 isolates Xv 80-6, Xv 81-23, and Xv 82-7, race 3 isolate Xv 77-3A, and tomato strain Xv 1926. The isolates had been stored in sterile water or frozen in glycerol. Inocula were prepared from agitated 24 h cultures from single colonies placed in sterilized nutrient broth. After centrifugation, the bacterial pellets were suspended in sterile tap water, and standardized colorimetrically to 50% light transmittance to approximate a density of 5×10^8 cfu ml^{-1}. These suspensions were either used directly or were serially diluted to final concentrations of approximately 10^3 to 3×10^3 cfu ml^{-1}, confirmed by replicated colony counts from 0.05 ml subsamples spread on nutrient agar plates. Virulence of all isolates was confirmed in Early Calwonder (ECW) plants. Correct race designation was confirmed by reaction on pepper lines following infiltration with 5×10^8 cfu ml^{-1} inoculum, as shown in Table 5-1. All inoculations were by hypodermic infiltration of intercostal leaf tissues.

Electrolyte Loss From Inoculated Leaf Tissues

The electrolyte loss over time as a measure of hypersensitive collapse of inoculated leaf tissues was determined. Plants of XVR 3-25, ECW, and its near isogenic line 10 R with the Bs_1 gene (Dahlbeck et al., 1979) were grown in a greenhouse before moving them to two controlled temperature chambers set at 30 ± 0.25 C, and 25 ± 0.25 C with 18 hour day length supplied by fluorescent and incandescent lamps. Areas about 4 to 5 cm^2 in three fully expanded leaves below the first fork

Table 5-1. Reaction of pepper lines and tomato to inoculation with races of *Xanthomonas campestris* pv. *vesicatoria*.

Race[a] designation	Reaction			
	Host line or cultivar			
Pepper strain	Early Calwonder	10 R	PI 271322 selection 271-4	Tomato
Race 1	Water-soaking and necrosis in 2-4 days	Water-soaking and necrosis in 2-4 days	Confluent HR[b] in 24 h	Water-soaking and necrosis in 2-4 days
Race 2	As above	Confluent HR in 12-18 h	Confluent HR in 18-24 h	As above
Race 3	As above	Water-soaking and necrosis in 3-4 days	Dry necrosis[c] in approx. 2 days; not confluent in 24 h	As above
Tomato strain	HR in 12-18 h	HR in 12-18 h	As above for race 3	As above

[a]Inoculum concentration 5×10^8 cfu ml^{-1}.

[b]HR = hypersensitive reaction.

[c]Neither HR nor susceptible necrosis following water soaking; an intermediate reaction.

were each inoculated with 5 x 10^8 cfu ml^{-1} inoculum of the same races and isolates used in the study of allelism of genes, and plants returned to their temperature treatments. Electrolyte losses were determined on 3.0 cm^2 samples of leaf tissue harvested at timed intervals after inoculation. Samples were suspended in 3.0 ml deionized water and conductivity in µmho of the suspending solutions was recorded immediately. Conductivity was again recorded after vacuum infiltration at 63 cm Hg for 60 seconds followed by agitation for 1 h at 30 C. The difference in conductivity was taken to represent influence of bacteria on host tissue (Cook and Stall, 1968).

Allelism of Genes

A selection of PI 271322, designated 271-4, was shown by progeny tests to be homozygous for both genes $\underline{Bs_3}$ and $\underline{Bs_1}$ that provide hypersensitivity to races 1 and 2, and for resistance to race 3 of Xcv (Chapter 3, this dissertation). A single plant of XVR 3-25, which is homozygous for genes that control hypersensitive resistances to races 1 and 2 (Cook, 1984), was included in the tests. The susceptible cultivar ECW and its near isogenic line, 10 R, were used as check lines.

A single plant of 271-4 was cross-pollinated with one plant of XVR 3-25. A single F_1 plant was then cross-pollinated with one of ECW to produce test-cross progeny seed. Segregation of dominant hypersensitivity genes was expected in the test-cross progeny as follows. A single allelic gene in both parents would result in hypersensitivity in all F_1 and test-cross progeny plants. A ratio of 3 hypersensitive plants: 1 nonhypersensitive plant would result if each parent carries a single but

different, independent HR gene. A 1:1 ratio is expected if a single HR gene is present in only one parent. Populations of 414 test-cross progeny plants, 30-40 plants of each parent and F_1 progeny, and 8 of line 10 R were planted in randomized rows of 8 plants in natural light in a greenhouse with temperature of 21 to 32 C. Plants were raised in 10-cm plastic pots with steam-sterilized peat-vermiculite mix, watered as required, and fertilized four times during the experiment with 0.4 g per pot of soluble 20:20:20 fertilizer.

The first and second, and seventh and eighth leaves from the cotyledons were infiltrated to observe the presence of hypersensitive responses to an isolate of each of three races in a repeated test. Several plants were tested three or four times to be sure of their response, or for demonstration purposes. To observe segregation for resistance not typically hypersensitive in nature to race 3, segments of the third and fourth leaves of all plants were infiltrated with a low concentration of 1.3×10^3 cfu ml^{-1} of the same race 3 isolate used for observing the presence or absence of hypersensitivity. The fifth and sixth leaves of a random sample of plants of parents, F_1, and 68 test-cross progeny, and line 10R were infiltrated with 2.7×10^3 cfu ml^{-1} of isolate Xv 80-5 of race 1 to correlate lesion numbers and diameters with the presence of hypersensitivity to that isolate. Leaves infiltrated with low concentration inoculum were harvested after three weeks. The number of discreet lesions developed in 2 cm^2 within a perimeter imprinted a cork borer were counted on each inoculated leaf viewed under a dissecting microscope (magnification, 2.5 X). The diameters of five lesions at random, or of all lesions where fewer than five existed, were measured on each leaf using a graduated eyepiece. Mean values were

obtained for each plant. The presence of hypersensitivity to nine additional Xcv isolates of all three races of the pepper strain and one of the tomato strain was tested on 20 additional plants of XVR 3-25 grown in the greenhouse.

Results and Discussion

Reaction of XVR 3-25 to Pepper and Tomato Strains of Xcv

Host tissue collapsed hypersensitively with all Xcv isolates in all XVR 3-25 plants. Hypersensitive reactions to all isolates of races 1 and 3 of the pepper strain, and the tomato strain were very similar in appearance, and lacked complete desiccation in the variable greenhouse environment. The HR to race 2 isolates was more complete necrosis and desiccation resembling HR to this race in 10 R and 271-4, or HR to race 1 in 271-4 (Cook and Guevara, 1982).

Electrolyte Loss From Inoculated Leaf Tissue

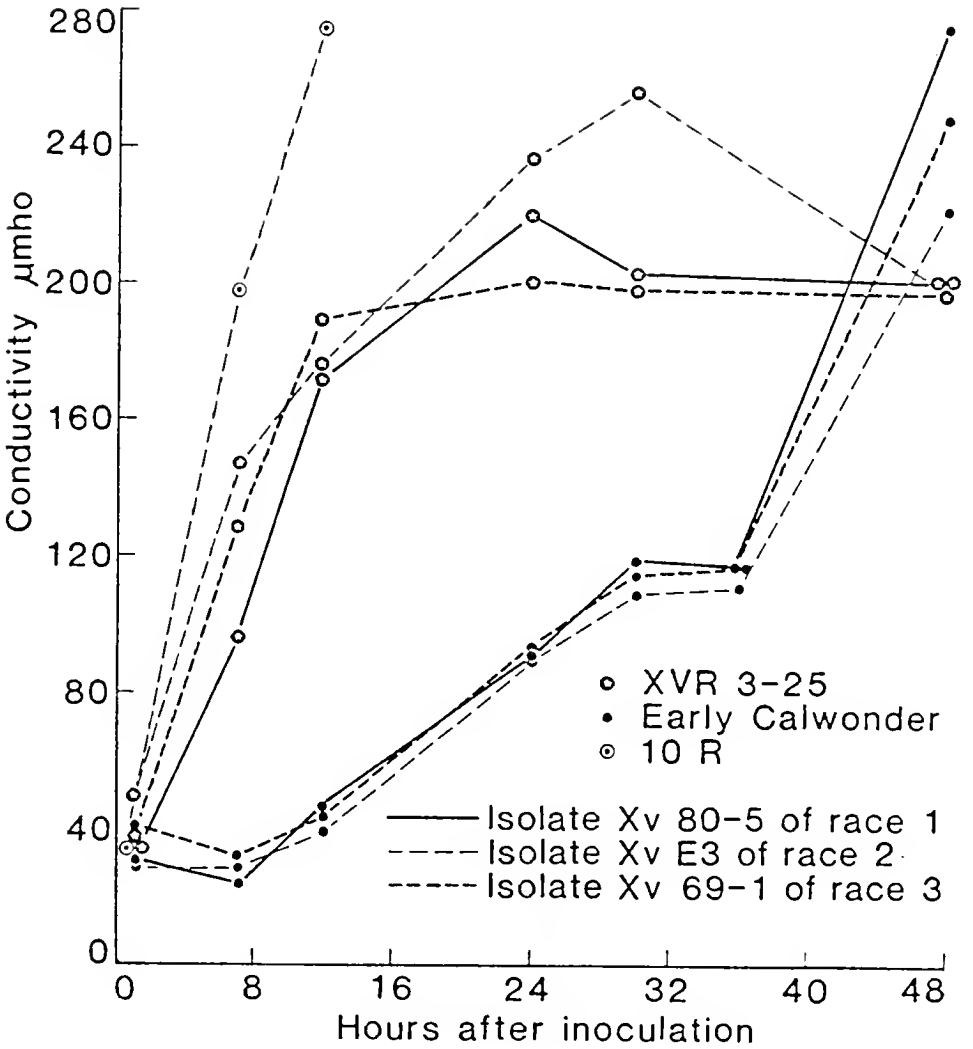
Mean values of electrical conductivity were computed for each sampling time in both temperature treatments from leaf discs of XVR 3-25, ECW, and 10 R inoculated with various isolates (Table 5-2). Values averaged across temperature treatments are shown in Figure 5-1 for each host-bacterial isolate combination. Conductivity increased slowly for all isolates in ECW tissues, with greater increases occurring at 30 C. Complete hypersensitive necrosis, reflected in rapid increase in conductivity, occurred within 12 h in line 10 R at both temperatures, and at 30 C in XVR 3-25 inoculated with race 2 isolate Xv E3. Hypersensitive necroses in XVR 3-25 were complete within 24 h with all three isolates in both temperature treatments, and were reflected in conductivity measurements. Conductivity increased more rapidly in XVR 3-25 at

Table 5-2. Conductivity as a measure of electrolyte loss at two temperatures from pepper leaf tissues inoculated with races 1, 2, and 3 of Xanthomonas campestris pv. vesicatoria.

Host variety	Xcv isolate	Hours after inoculation[a]						
		1	7	12	24	30	36	48
		Conductivity in µmho						
XVR 3-25	Xv 80-5	35[b]	111	121	203	215	-	267[c]
	(race 1)	35	81	225	236	215	-	137
	Xv E3	68	203	229	266	-	-	223
	(race 2)	33	92	121	206	256	-	171
	Xv 69-1	43	159	160	217	217	-	225
	(race 3)	33	96	219	185	180	-	170
Early Calwonder	Xv 80-5	28	25	48	110	149	150	351
	(race 1)	39	24	47	63	89	82	198
	Xv E3	27	40	42	115	131	135	255
	(race 2)	30	20	37	67	86	89	189
	Xv 69-1	43	44	48	112	145	129	261
	(race 3)	38	21	41	67	85	107	214
10 R	Xv E3	34	234	252	-	-	-	-
	(race 2)	34	160	298	-	-	-	-

[a]Inoculum concentration approximately 5×10^8 cfu ml^{-1}
[b]Upper value 30 C temperature; lower value 25 C; means of 6 replicates
[c]Mean of 3 or 6 replicates at 48 h

Figure 5-1. Conductivity as a measure of electrolyte loss from pepper leaf tissues inoculated with three races of Xanthomonas campestris pv. vesicatoria. Points represent means of 12 replicates except those of cv. XVR 3-25 at 48 h which represent means of 6 replicates.


280
240
200
160
120
80
40
0
Conductivity µmho
0
8
16
24
32
40
48
Hours after inoculation
XVR 3-25
Early Calwonder
10 R
Isolate Xv 80-5 of race 1
Isolate Xv E3 of race 2
Isolate Xv 69-1 of race 3

25 C than at 30 C with races 1 and 3 isolates Xv 80-5 and Xv 69-1, respectively.

Electrolyte losses from XVR 3-25 were consistent with HR to all three races of the pepper strain of Xcv. The HR to race 2 isolate Xv E3 was similar in both line 10 R and XVR 3-25. Cook and Guevara (1984) demonstrated that hypersensitivity in a progenitor of XVR 3-25 was associated with far fewer bacteria than in a susceptible host, so bacterial populations were not followed here.

Allelism of Genes for Hypersensitive Resistances

All plants of the parents 271-4 and XVR 3-25, their F_1 and test-cross progeny (F_1 x ECW), and check line 10 R, but none of susceptible check cultivar ECW were hypersensitive to isolate Xv E3 of race 2 in each of two separate tests (Table 5-3). These data are consistent with one allelic dominant gene present in homozygous condition in both parents 271-4 and XVR 3-25. Only one dominant gene $\underline{Bs_1}$ for HR to race 2 has been observed in segregating progeny derived from 271-4 (Chapter 3, this dissertation), and Cook (1984) reported that XVR 3-25 also contained the $\underline{Bs_1}$ gene from cultivar Florida VR-2.

All plants of both parents 271-4 and XVR 3-25 and their F_1 progeny, but none of check lines 10 R and ECW were hypersensitive to race 1 isolate Xv 80-5 in two separate tests (Table 5-3). The test-cross progeny segregated with 318 hypersensitive: 96 nonhypersensitive, consistent with a 3:1 ratio (chi^2 = 0.72, P = 0.50-0.30) expected with two independently segregating dominant genes, one present in homozygous condition in each of 271-4 and XVR 3-25.

Table 5-3. Segregation for hypersensitive reactions in a pepper test-cross population inoculated with races 1, 2, and 3 of *Xanthomonas campestris* pv. *vesicatoria*.

Host population	Genotype	Reaction[a]					
		Race 1 isolate Xv 80-5		Race 2 isolate Xv E3		Race 3 isolate Xv 69-1	
		HR[b]	non-HR	HR	non-HR	HR	non-HR
271-4	Bs_1 Bs_3	30	0	30	0	0	30
XVR 3-25	Bs_1 Bs_2	35	0	35	0	35	0
F_1 (271-4 x XVR 3-25)		40	0	40	0	40	0
F_1 x Early Calwonder		318	96	414	0	208[c]	206[d]
Early Calwonder	bs_1 bs_2 bs_3	0	40	0	40	0	40
10 R	Bs_1 bs_2 bs_3	0	8	8	0	0	8

[a]Inoculum concentration approximately 5×10^8 cfu ml^{-1}
[b]HR - hypersensitive reaction
[c]Also all were HR to race 1; but of 208 plants, 102 had HR typical of XVR 3-25 and 106 had HR typical of 271-4 with race 1.
[d]Also 110 had HR to race 1 typical of 271-4, and 96 had non-HR to race 1.

Typical hypersensitivity to race 3 isolate Xv 69-1 occurred in all plants of both XVR 3-25, and the F_1 progeny of 271-4 x XVR 3-25, but in none of 271-4 and susceptible checks ECW and 10 R (Table 5-3). The test-cross progeny segregated with 208 hypersensitive: 206 nonhypersensitive in two tests, consistent with a 1:1 ratio expected with a single dominant gene for HR present in homozygous condition only in XVR 3-25. The necrosis in all 208 test-cross plants with HR appeared very similar or identical to the HR in XVR 3-25 with the same isolate.

Hypersensitivity to races 1 and 3 of Xcv did not segregate independently in test-cross progeny. The 208 plants hypersensitive to race 3 isolate Xv 69-1 were also hypersensitive to race 1 isolate Xv 80-5. Necroses similar to those in XVR 3-25 and 271-4 plants occurred in, respectively, 102 and 106 of these test-cross progeny with race 1 isolate Xv 80-5. The 206 plants nonhypersensitive to Xv 69-1 of race 3 segregated with 110 hypersensitive: 96 nonhypersensitive to Xv 80-5 of race 1 and all 110 hypersensitive plants had the HR necrosis typical of the 271-4 parent. The combined segregation pattern is consistent (chi^2_{2df} = 0.82, P = 0.7-0.5) with a single, homozygous, dominant gene in XVR 3-25 for HR to both races 1 and 3, and a different, independent dominant gene in 271-4 for HR to race 1 only.

Only a few lesions of small diameter occurred with Xv 69-1 of race 3 in plants of both 271-4 and XVR 3-25, their F_1 progeny, and in all 208 hypersensitive test-cross progeny plants three weeks after infiltrating leaves with 1.3 x 10^3 cfu ml^{-1} inoculum (Table 5-4). In contrast, many relatively large lesions occurred in plants of ECW and line 10 R. Wide variation in numbers and diameters of lesions occurred amongst the 206

Table 5-4. Number of lesions per 2 cm^2 of leaf and diameter per lesion in parents, F_1, test-cross progeny and check line 10R inoculated with race 3 isolate Xv 69-1 of Xanthomonas campestris pv. vesicatoria.

Generation	Plants hypersensitive			Plants non-hypersensitive		
	Number	Lesions[a] per 2 cm^2	Diameter per lesion (mm x 10)	Number	Lesions per 2 cm^2	Diameter per lesion (mm x 10)
271-4	-	-	-	30	0.30 ± 0.13[b]	1.25 ± 0.056
XVR 3-25	35	0.40 ± 0.12	1.33 ± 0.250	-	-	-
F_1 (271-4 x XVR 3-25)	40	0.50 ± 0.04	1.15 ± 0.150	-	-	-
F_1 x Early Calwonder	208	0.12 ± 0.02	1.55 ± 0.157	206	14.6 ± 0.45	3.79 ± 0.127
Early Calwonder	-	-	-	40	16.6 ± 0.58	5.30 ± 0.330
10 R	-	-	-	4	19.0 ± 3.32	6.80 ± 1.090

[a]Inoculum concentration 1.3 x 10^3 cfu ml^{-1}
[b]Mean ± standard error of mean.

nonhypersensitive test-cross progeny plants, but most had many lesions of relatively large diameter (Table 5-4), and no discreet segregation ratio was apparent. These data are consistent with an incompletely recessive gene for resistance to this race from 271-4 (Stall, 1981; Chapter 3 of this dissertation). Hypersensitivity from XVR 3-25 was epistatic to the expression of resistance to isolate Xv 69-1 of race 3 from 271-4. The means and ranges in numbers and diameters of lesions with isolate Xv 69-1 in nonhypersensitive test-cross progeny plants did not differ significantly for those 110 plants with HR and the 96 plants without HR to isolate Xv 80-5 of race 1.

The sample of 68 test-cross progeny infiltrated with 2.7×10^3 cfu ml^{-1} of isolate Xv 80-5 of race 1 comprised 15 plants hypersensitive to races 1 and 3, 39 plants nonhypersensitive to both races, and 14 plants hypersensitive to race 1 but nonhypersensitive to race 3. Few lesions of small diameter occurred on plants hypersensitive to isolate Xv 80-5 in contrast to relatively high mean values in both line 10 R, and non-hypersensitive test-cross plants (Table 5-5). An exceptional test-cross plant was found, however, which, although previously classified as hypersensitive to both races 1 and 3, possessed means of 7.5 lesions per 2 cm^2 of leaf with race 1 isolate Xv 80-5, and zero lesions with race 3 isolate Xv 69-1. This plant was, however, clearly less susceptible to race 1 isolate Xv 80-5 than the mean of all sampled test-cross plants classified nonhypersensitive to race 1 (Table 5-5).

Wide variation in number of lesions per 2 cm^2 of leaf and lesion diameter occurred for both isolates Xv 80-5 (race 1) and Xv 69-1 (race 3) in the sample of 39 test-cross progeny plants lacking genes for HR to

Table 5-5. Number of lesions per 2 cm^2 of leaf and diameter per lesion in samples of plants of parents, F_1, test-cross progeny, and check line 10 R inoculated with race 1 isolate Xv 80-5 of *Xanthomonas campestris* pv. *vesicatoria*

Generation	Plants hypersensitive			Plants nonhypersensitive		
	Number Sampled	Lesions[a] per 2 cm^2	Diameter per lesion (mm x 10)	Number Sampled	Lesions per 2 cm^2	Diameter per lesion (mm x 10)
271-4	5	0.0	-	-	-	-
XVR 3-25	5	0.2 ± 0.14[b]	2.0 ± 0	-	-	-
F_1 (271-4 x XVR 3-25)	6	0.5 ± 0.25	1.0 ± 0	-	-	-
F_1 x Early Calwonder	29	0.7 ± 0.28	1.5 ± 0.25	39	25.3 ± 1.97	6.0 ± 0.16
10 R	-	-	-	3	38.0 ± 1.97	6.6 ± 0.19

[a]Inoculum concentration approximately 2.7 x 10^3 cfu ml^{-1}
[b]Mean ± standard error of mean

both races. The two attributes were converted to a mean diseased area per 2 cm^2 of leaf for each plant, based on the generally valid observation of circular lesions. Diseased areas were positively correlated between isolates ($r=0.56$, $P<0.05$), and plants either simultaneously susceptible or resistant to both isolates were identified. The genes for hypersensitivity to race 1 in 271-4 and XVR 3-25 were epistatic to the expression of nonhypersensitive resistance to race 1.

Hypersensitive resistance is known to be epistatic to other forms of resistance (Johnson, 1983; but note Clifford, 1974). Since all test-cross progeny reacted with hypersensitivity to race 2 isolate Xv E3, no possible correlation of nonhypersensitive resistance between isolates of race 2 and races 1 and 3 could be detected. Nonhypersensitive resistance in 271-4 to race 3 isolate Xv 69-1 may be generalized against all races (Stall, 1981; Chapter 3 of this dissertation).

Races of the pepper strain of Xcv are separated on pepper lines with genes for hypersensitivity from C. annuum (Cook and Stall, 1969; Chapter 3 of this dissertation). The Bs_2 gene in XVR 3-25 from C. chacoense PI 260435 did not distinguish between races 1 and 3, and may possibly also be effective against race 2. Previously (Kim and Hartmann, 1985), the Bs_2 gene was thought to be effective against both races 1 and 2. This could not be tested in this experiment since both parents, 271-4 and XVR 3-25, carry the Bs_1 gene for HR to race 2.

A single test-cross progeny plant was located which did not fully fit within expected classifications of hypersensitivity when tested against low inoculum concentrations. It is possible the Bs_2 locus from XVR 3-25 may contain more than one gene, i.e., close linkage or even a

compound locus which may be subject to a low frequency of genetic recombination. For practical breeding purposes the locus was effective here against isolates of races 1 and 3.

The line XVR 3-25 reacted consistently with 12 isolates of three races of Xcv pathogenic on pepper, and with one of the tomato strain. Durability of resistance from these genes, however, cannot be predicted.

CHAPTER 6
INHERITANCE OF RESISTANCE TO BACTERIAL SPOT IN PEPPER PLANT INTRODUCTION 163189

Bacterial leaf spot, incited by Xanthomonas campestris pv. vesicatoria (Doidge, 1920), Dye 1978 (designated as Xcv) is the most destructive foliar disease of bell pepper (Capsicum annuum L.) in warm humid climates. There have been three races of the pepper strain pathogenic on pepper differentiated by inoculation of plants with the dominant genes, Bs_1 and Bs_3, for hypersensitive resistances to races 2 and 1, respectively (this dissertation, Chapter 4). These genes were found in plants of plant introductions (PI) 163192 (Cook and Stall, 1963), and PI 271322 (Kim and Hartmann, 1985), respectively. The released bell pepper Florida VR-4 (Cook, 1984) has the Bs_1 gene (A. A. Cook, 1982, personal communication).

The resistance in PI 163189 has proven durable (Adamson and Sowell, 1983; Hibberd et al., 1979; Sowell, 1960; Sowell and Dempsey, 1977). This line was one of several successfully used in breeding resistant bell pepper during the 1960's (Borchers, 1965). Resistant segregates were selected from field grown, naturally infected inbred backcross progenies. By implication this resistance was not inherited as a dominant trait, but as a recessive. Useful genetic gains were made, but the program was discontinued and seed is no longer available (E.A. Borchers, 1978, personal communication). Cook and Stall (1969) observed heterogeneity for hypersensitivity to race 2 in PI 163189, but all plants lacked typical hypersensitivity to race 1. Adamson and

Sowell (1983) presented inconclusive evidence to support a two-gene system for resistance to race 2, in PI 163189 namely, a dominant gene linked to $\underline{Bs_1}$, and a second, possibly independent, dominant gene.

I hypothesized that more than one gene does exist in PI 163189 to provide hypersensitive and non-hypersensitive resistances. This chapter presents evidence that supports a model of two independent genes in a homozygous selection derived from PI 163189.

Materials and Methods

Inoculum Preparation

The Xcv isolates used in these studies were from cultures stored frozen in 15% glycerol or in refrigerated sterilized water. Inocula were prepared from 24 h, agitated, late log-phase, cultures derived from single colonies in sterile nutrient broth. After centrifugation, bacterial pellets were resuspended in sterile tap water, and standardized colorimetrically to 50% light transmittance to approximate a density of 5×10^8 cfu (colony forming units) ml^{-1}. These suspensions were either used directly for observing HR, or serially diluted to final concentrations of approximately 1×10^3 to 3×10^3 cfu ml^{-1}, confirmed by replicated colony counts from 0.05 ml subsamples spread on nutrient agar plates. Virulence of all isolates was confirmed in plants of the susceptible cultivar Early Calwonder (ECW). Race designation was confirmed by reaction of different pepper lines following infiltration with 5×10^8 cfu ml^{-1} inoculum (this dissertation, Chapters 3 and 4).

Heterogeneity of Resistance in PI 163189

An estimate of the variability in reactions of PI 163189 to isolates of three races of Xcv was obtained from a small population.

Plants of this line, the susceptible control ECW, and its near isogenic line, 10 R, with the $\underline{Bs_1}$ gene (Dahlbeck et al., 1979), were raised in steamed peat-vermiculite mix in 10-cm plastic pots arranged in a greenhouse (temperature range 20 to 35 C). Rows of eight plants were randomized. Plants were watered as required and treated four times during the experiment with approximately 0.4 g per pot of soluble 20:20:20 fertilizer. Four fully expanded leaves of PI 163189 and ECW were each infiltrated with approximately 3×10^3 cfu ml^{-1} of each of four isolates, Xv 0623 and Xv 82-8 (race 1), Xv 82-7 (race 2), and Xv 77-3A (race 3). A single leaf was sampled from each plant at 11, 14, 21, and 35 days following infiltration. The numbers of lesions per 2 cm^2 of leaf within a perimeter imprinted by a cork-borer was counted at each inoculation site viewed under a dissecting microscope (magnification 2.5X). The diameters of five random lesions per site, or of all lesions where fewer than five existed, were measured at day 35 using a graduated eyepiece. Inoculum of each isolate containing approximately 5×10^8 cfu ml^{-1} was infiltrated into two additional leaves per plant of PI 163189, ECW, and 10 R, and plants observed after 24 h for hypersensitivity (Cook and Stall, 1969). A single plant of PI 163189, designated 189-5 was selected for hypersensitivity to race 2 and resistance to races 1 and 3. Inbred progeny of 189-5 were used in subsequent experiments.

Electrolyte Loss from Inoculated Leaf Tissues

Electrolyte loss from inoculated leaf tissue was used to characterize hypersensitivity to race 2 in 189-5. Losses were determined on 3.0 cm^2 samples of leaf tissue harvested at timed intervals after

inoculation with 5 x 10^8 cfu ml^{-1} of isolate Xv E3 and sterile water. Plants were kept at constant 26.5 ± 0.25 and 28.5 ± 0.25 C. Samples were suspended in 3.0 ml deionized water and conductivity in µmho of the suspending solution was recorded immediately. Conductivity was again recorded after vacuum infiltration for 60 seconds at 63 cm Hg and by agitation for 1 h at 30 C. The difference in conductivity was taken to represent the influence of bacteria on host tissue (Cook and Stall, 1968).

Bacterial Populations in vivo

Low populations of races 1, 2, and 3 in plants of 189-5 were expected to correlate with a disease severity lower than in susceptible control plants. Plants of 189-5, ECW, and 10R were raised in a greenhouse as described above. Three fully expanded leaves per plant were each infiltrated with three Xcv isolates: Xv 80-5 (race 1, 1.4 x 10^3 cfu ml^{-1}), Xv E3 (race 2, 2.2 x 10^3 cfu ml^{-1}), and Xv 69-1 (race 3, 0.8 x 10^3 cfu ml^{-1}). Leaves from single inoculated plants were harvested periodically after inoculation, namely, at 0, 2, 5, 8, 11, and 14 days. Bacterial populations were determined from replicated 1.0 cm^2 (i.e., 2 x 0.5 cm^2) leaf samples. Samples were triturated in 0.5 ml sterile water, the suspension serially diluted when appropriate, and 0.05 ml subsamples of the final dilution spread on nutrient agar plates. Colonies of Xcv were counted after 2 to 3 days incubation at 30 C, and mean values converted to $\log_{10}$(cfu cm^{-2}) of leaf. The numbers of lesions per 2 cm^2 and diameters of a maximum of 5 lesions per inoculation site were also obtained on additional plants 15 days after inoculation.

Inheritance of Hypersensitive and Non-hypersensitive Resistances

The homozygous selection, 189-5, with hypersensitivity to isolates of race 2 and non-hypersensitive resistance to isolates of races 1 and 3 was used. Florida VR-4 (with the $\underline{Bs_1}$ gene) and check control lines ECW and 10 R also were used. A single plant of Florida VR-4 was cross-pollinated with one of 189-5. A single F_1 plant was self-pollinated to yield F_2 seed and also cross-pollinated with an additional plant of both parents to give backcross progenies.

Populations of 561 F_1 seedlings, 40 to 50 of each of the two backcrosses, 30 to 40 of both 189-5 and the F_1 progeny, and 10 each of Florida VR-4, line 10 R, and ECW were raised in seedling flats in a greenhouse (temperature range 21 to 35 C). The following inoculations were applied to all eight populations. When cotyledonary leaves of the seedlings were fully expanded, one cotyledon on each plant was inoculated with isolate Xv 82-7 of race 2. Inoculum was infiltrated using sterilized 27-gauge hypodermic needles and syringes. Inoculum density was 5×10^8 cfu ml^{-1}. Hypersensitivity was observed 24 h after inoculation (Cook and Stall, 1969). On completion of that test, seedlings were transplanted to pots as described above. Space limitations reduced the F_2 population to 282 seedlings.

Isolate Xv 82-7 of race 2 was used a second time to inoculate all seedlings at a more mature stage. A leaf adjacent to the first fork of the stem was infiltrated with inoculum at 5×10^8 cfu ml^{-1} and observed for hypersensitive tissue collapse within 24 h. To observe nonhypersensitive resistances the preceding three leaves were each infiltrated with low concentration (approximately 2×10^3 cfu ml^{-1}) of each of the

isolates Xv 82-8 (race 1), Xv 82-7 (race 2) and Xv 77-3A (race 3). Overlapping of watersoaked infiltrated areas was avoided. These leaves were harvested after 14 days, sealed in plastic bags and refrigerated (3 C). Over the next several days the number of discreet lesions per 2 cm^2 of leaf and diameters of a maximum of five lesions were obtained for each inoculation site as described above. Mean values for lesion numbers and diameter were obtained for each isolate on each plant.

Hypersensitivity to race 2 was expected to be a qualitative trait (Cook and Stall, 1969). Nonhypersensitive resistance assessed by present methods is quantitative, and may not result in discreet classes of resistant and susceptible plants in segregating populations (Adamson and Sowell, 1983; Hibberd and Gillespie, 1982a; Stall, 1981). In that event, data were evaluated by analysis of generation means weighted by their variances (Basford and De Lacy, 1979; Hayman, 1958; Mather and Jinks, 1971). The matrix specification of Fisher (1918) was used, with parameters: $\underline{m}$ = general theoretical population mean at F_∞; $\underline{a}$ = value of additivity; $\underline{d}$ = value of dominance deviation. The analysis applies a chi^2 goodness of fit test to computed parameters for a simple, single gene model. In the event of poor fit, the analysis proceeds to fit a digenic model with interactions (Basford and De Lacy, 1979). Specific comparisons of means were performed by t-test.

Results and Discussion

Heterogeneity of Resistance in PI 1673189

Significantly fewer lesions per 2 cm^2 of leaf occurred in PI 163189 plants than in ECW plants with all isolates following

infiltration with low inoculum concentration (Table 6-1). Lesions in ECW plants were approximately six times the diameter of those in PI 163189 plants at day 35. Wide ranges in lesion numbers occurred among PI 163189 plants with each isolate, but little variation occurred in lesion diameter and all plants appeared resistant. Four of nineteen plants of PI 163189 had very few lesions with any isolate.

Hypersensitivity to isolate Xv 82-7 of race 2 occurred in 6 of 19 plants of PI 163189 within 24 h after inoculation with 5×10^8 cfu ml^{-1}. The reaction in leaves of 10 R with the same isolate was identical. Necrosis in 2.5 to 3 days preceded by extensive watersoaking occurred in leaves of ECW with all isolates, and in 10 R with isolates of races 1 and 3. Necrosis in 2 to 3 days, not preceded by extensive watersoaking occurred in 13 of 19 plants of PI 163189 with isolate Xv 82-7, and in all 19 plants with isolates of races 1 and 3. This reaction was distinguishable from HR by developing later, and from the susceptible reaction by the absence of extensive watersoaking. Plants of PI 163189 which were hypersensitive and nonhypersensitive to isolate Xv 82-7 of race 2 had similar numbers of lesions with all isolates. The plant designated 189-5 was selected for hypersensitivity to isolate Xv 82-7, and for very few, small lesions with isolates of races 1 and 3. Progeny from self-pollinating this plant were uniform for these two resistance reactions, and the line was used in subsequent experiments.

Table 6-1. Lesions per 2 cm^2 of leaf at four sampling times and mean diameter per lesion 35 days after inoculation of peppers with races 1, 2, and 3 of Xanthomonas campestris pv. vesicatoria.

Bacterial isolate			Lesions per 2 cm^2 of leaf[a] Days after inoculation				Diameter per lesion (mm)
Designation	Race	Host	11	14	21	35	at day 35
Xv 0623	1	PI 163189[b]	2.2 ± 0.71[c]	6.6 ± 1.37	7.4 ± 1.67	8.9 ± 2.06	0.17 ± 0.01
		ECW[d]	3.0 ± 1.63	6.7 ± 2.04	24.3 ± 3.76	Confluent[e]	1.25
Xv 82-8	1	PI 163189	7.2 ± 1.03	14.1 ± 2.43	15.7 ± 3.14	27.1 ± 4.57	0.17 ± 0.01
		ECW	17.0 ± 4.6	26.8 ± 3.59	38.3 ± 2.90	Confluent	1.00
Xv 82-7	2	PI 163189	1.5 ± 0.50	2.3 ± 0.79	4.1 ± 1.33	4.71 ± 1.51	0.14 ± 0.01
		ECW	3.2 ± 1.59	8.5 ± 3.47	22.7 ± 4.22	Confluent	1.03
Xv 77-3A	3	PI 163189	1.6 ± 0.53	4.7 ± 1.17	6.6 ± 1.54	10.11 ± 2.75	0.15 ± 0.01
		ECW	2.2 ± 0.94	5.6 ± 2.65	22.3 ± 5.55	Confluent	0.88
General Mean		PI 163189	3.1	7.4	8.4	12.7	0.16
		ECW	6.4	11.9	26.9	Confluent	1.04

[a]Inoculum concentration approximately 3 x 10^3 cfu ml^{-1}.
[b]Means are from 19 and 6 plants of PI 163189 and Early Calwonder, respectively.
[c]Mean ± standard error of mean.
[d]ECW = Early Calwonder.
[e]Confluent necrosis; lesions were difficult to count.

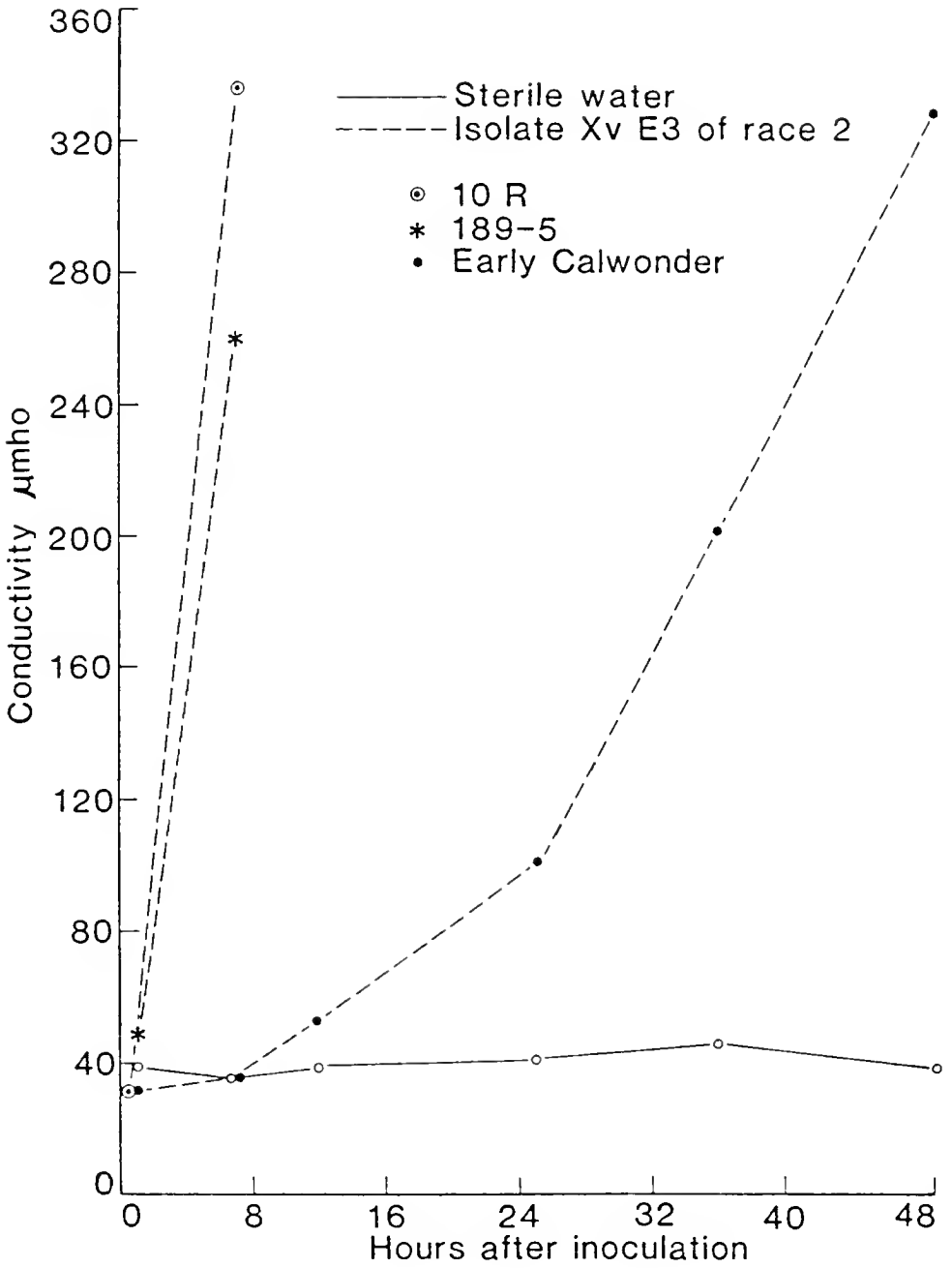
The frequency of hypersensitivity in PI 163189 to isolates of race 2 was similar to that observed by Cook and Stall (1969). This implied that the line had altered little in the intervening years and that a determination of inheritance of resistance might depend on the parent plant chosen.

Hypersensitivity was detected by infiltrating a high inoculum concentration (Cook and Stall, 1969) so that all host cells in the inoculated zone responded (Klement et al., 1964; Klement, 1982; Stall and Cook, 1966). Nonhypersensitive resistance was detected more easily by examining two components of resistance, namely numbers of lesions per unit area (Stall, 1981), and lesion diameter following infiltration with low inoculum concentration. Previously (Cook and Stall, 1969), nonhypersensitive necrosis in PI 163189 after inoculating with high concentrations of bacteria was regarded as indistinguishable from the reaction of susceptible control cultivars. Clearly, the line was not susceptible in this and other greenhouse and field experiments (Adamson and Sowell, 1983; Borchers, 1965; Hibberd et al., 1979; Sowell and Dempsey, 1977). As a consequence, opinions diverged over the value of infiltrating high inoculum concentrations (Sowell and Dempsey, 1977; G. Sowell Jr., 1978, personal communication). The effect of a gene controlling HR is most efficiently observed by infiltrating high inoculum density (Cook and Stall, 1968).

Electrolyte Loss from Inoculated Leaf Tissues

Electrical conductivity of the solution bathing discs from leaves infiltrated with 5×10^8 cfu ml^{-1} of isolate Xv E3 of race 2 increased gradually in ECW (Figure 6-1) and more rapidly at 28.5 than 26.5 C

Figure 6-1. Electrical conductivity as a measure of electrolyte loss from leaf tissue of peppers inoculated with sterile water and race 2 of Xanthomonas compestris pv. vesicatoria. Points with isolate Xv E3 represent means of 6 replicates, and water treatment points represent means of 18 replicates averaged for all three pepper hosts.


360
320
280
240
200
160
120
80
40
0
Conductivity µmho
0
8
16
24
32
40
48
Hours after inoculation
Sterile water
Isolate Xv E3 of race 2
10 R
189-5
Early Calwonder

(Table 6-2). Visible leaf tissue collapse occurred between 36 and 49 h, and this was asssociated with a large increase in conductivity. In contrast, conductivity in both temperature treatments increased greatly in 7 h after inoculation of both 189-5 and 10 R. Visible hypersensitive necrosis was complete by that time. The rate of increase in 189-5 was slightly greater at 28.5 than 26.5 C. Conductivity did not increase from leaf discs of any host infiltrated with sterile water (Figure 6-1). These data are entirely characteristic of hypersensitivity (Cook and Stall, 1968) which was essentially equal in both 189-5 and 10 R.

Bacterial Populations in vivo

Populations of bacteria of isolates of all three races in vivo reached approximately 5 x 10^7 to 10^8 cfu cm^{-2} in leaves of ECW 14 days after infiltration with low inoculum concentrations (Figure 6-2). In contrast, populations of all isolates in 189-5 leaves and of isolate Xv E3 of race in 10 R leaves plateaued between 2 and 5 days after inoculation at levels about 10^3 to 10^4 fewer than in ECW leaves.

Many lesions of relatively large diameter developed in leaves of ECW with all three isolates and in 10 R with isolates Xv 80-5 (race 1) and Xv 69-1 (race 3) (Table 6-3). Lesions became visible in 5-6 days after inoculation, corresponding to bacterial populations of 10^6 to 10^7 cfu cm^{-2}. Small necrotic hypersensitive flecks developed in leaves of 10 R with isolate Xv 82-7 of race 2. In contrast, the lesions in leaves of 189-5 were few in number per 2 cm^2 of leaf and had small diameters for all isolates tested.

Few lesions per 2 cm^2 of leaf and their smaller diameter per lesion together reflected low bacterial populations of races 1, 2, and 3 in

Table 6-2. Conductivity as a measure of electrolyte loss from pepper leaf tissue inoculated with race 2 of Xanthomonas campestris pv. vesicatoria and incubated at either of two temperatures.

Host	Temp (C)	Conductivity (μmho) Hours after inoculation[a] 1	7	12	25	30	49
ECW[b]	26.5	20[c]	30	54	81	145	308
	28.5	43	43	53	122	259	306
189-5	26.5	43	197	289	--[d]	--	--
	28.6	53	322	214	--	--	--
10 R	26.5	29	324	274	--	--	--
	28.5	36	309	179	--	--	--

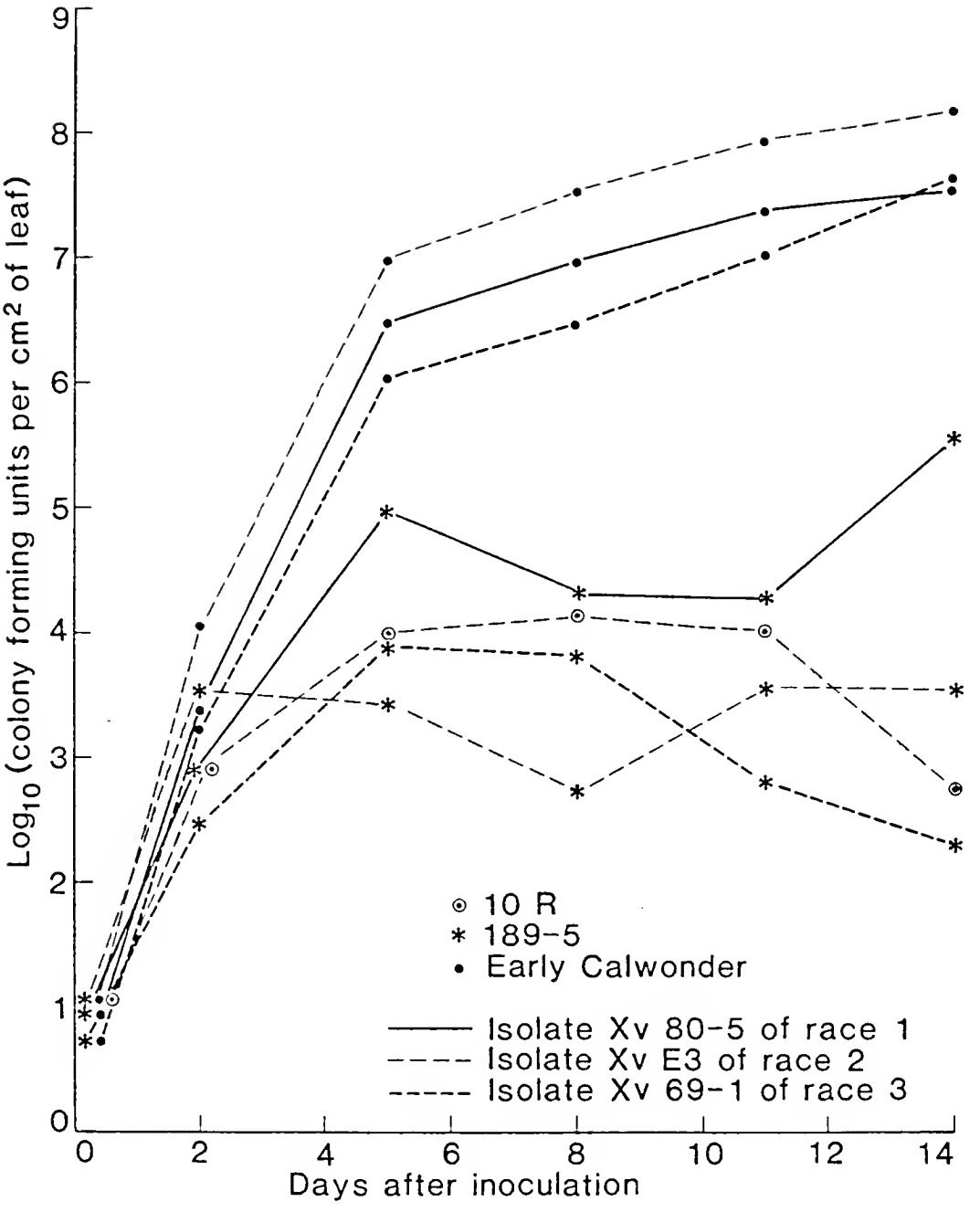
[a]Inoculum concentration 5 x 10^8 cfu ml^{-1} with isolate Xv E3.
[b]ECW = Early Calwonder.
[c]Values are means of three replicates.
[d]Data not collected; hypersensitive collapse judged complete within 12 h.

Table 6-3. Number of lesions per 2 cm^2 and diameter per lesion in leaves of 189-5, and check lines Early Calwonder and 10 R 15 days after inoculation with races 1, 2, and 3 of Xanthomonas campestris pv. vesicatoria.

Xcv isolate and race	Number of lesions[a] per 2 cm^2			Diameter per lesion (mm x 10)		
	Host			Host		
	189-5	ECW[b]	10 R	189-5	ECW	10 R
Xv 80-5 (race 1)	0.87 ± 0.54[c]	22.3 ± 1.27	24.7 ± 2.08	1.7 ± 0.41	6.9 ± 0.88	4.9 ± 0.33
Xv E3 (race 2)	0.40 ± 0.36	28.0 ± 1.67	5.7 ± 0.94	2.0 ± 1.41	8.2 ± 1.07	1.7 ± 0.29
Xv 69-1 (race 3)	0.20 ± 0.24	15.4 ± 1.71	11.1 ± 1.95	1.0 ± 0	7.5 ± 1.19	5.4 ± 0.82

[a]Inoculum concentration approximately 1.5 x 10^3 cfu ml^{-1}.
[b]ECW = Early Calwonder.
[c]Mean ± standard error of mean.

Figure 6-2. Bacterial populations in pepper leaves inoculated with isolates of races 1, 2, and 3 of Xanthomonas campestris pv. vesicatoria. Each point represents the mean of 3 replicates.


Log10 (colony forming units per cm2 of leaf)
0
1
2
3
4
5
6
7
8
9
0
2
4
6
8
10
12
14
Days after inoculation
⊙ 10 R
* 189-5
• Early Calwonder
Isolate Xv 80-5 of race 1
Isolate Xv E3 of race 2
Isolate Xv 69-1 of race 3

leaves of 189-5. These populations, and of isolate Xv E3 (race 2) in 10 R leaves, increased for several days after inoculation before reaching a plateau. This is a characteristic of hypersensitivity (Klement et al., 1964). Final populations of bacteria in these reactions were approximately equal. These data are consistent with a bacterial multiplication in resistant leaves being insufficient for necrotic areas to become visible. Necrotic areas became visible lesions following more extensive bacterial multiplication in the susceptible host.

Inheritance of Resistances

Isolate Xv 82-7 of race 2

Both cotyledonary and mature leaves were infiltrated with 5×10^8 cfu ml^{-1} of isolate Xv 82-7 of race 2. A hypersensitive reaction was observed at both maturity stages in plants of the parents 189-5 and Florida VR-4, their F_1 and F_2 progeny, the two backcrosses, and line 10 R, but in none of the susceptible check cultivar ECW. This result is consistent with one allelic dominant gene, $\underline{Bs_1}$, present in homozygous condition in both parents 189-5 and Florida VR-4. This is a plausible explanation of the linkage of resistance in PI 163189 and PI 163192 (a progenitor of Fla. VR-4) observed by Adamson and Sowell (1983). The gene $\underline{Bs_1}$ was clearly identified in cotyledons of homozygous (for that gene) young seedlings raised in trays. This test promises a more efficient use of labor and resources in selecting plants with $\underline{Bs_1}$ from segregating progenies than inoculating mature leaves.

A few necrotic hypersensitive flecks developed in hypersensitively resistant plants two weeks after infiltrating leaves with 1.1×10^3 cfu ml^{-1} of the same isolate (Table 6-4). In contrast, many more lesions of large diameter developed in plants of ECW. The generation means of numbers of hypersensitive flecks per 2 cm^2 varied (Table 6-4). Significantly more ($P < 0.05$) developed in leaves of Florida VR-4 than in any other generation or in check line 10 R, and no flecks were detected in leaves of 189-5. The means for other generations were intermediate between these extremes and appeared to reflect additive inheritance, i.e., as the frequency of alleles of "background" gene(s) from Fla. VR-4 increased, so did the numbers of hypersensitive flecks. The numbers of flecks were too low to warrant further analysis of generation means and variances. It is noteworthy, however, that such variation existed. Plants in this experiment excepting ECW were homozygous for $\underline{Bs_1}$ but may have varied in genetic background resistance to the pathogen (Clifford, 1974; Parlevliet, 1983).

Isolate Xv 82-8 of race 1 and isolate Xv 77-3A of race 3

Wide differences occurred among generations for the numbers of lesions per 2 cm^2 of leaf and diameter per lesion in leaves infiltrated with both isolates at low concentrations (Tables 6-5, 6-6, 6-7, and 6-8). Few lesions of small diameter occurred in leaves of 189-5 in contrast to many more, large lesions in Fla. VR-4, and in control lines ECW and 10 R. Lesions with isolate Xv 82-8 of race 1 were consistently of greater diameter in all generations than with isolate Xv 77-3A of race 3.

The F_1 and backcross (F_1 x Fla. VR-4) populations appeared susceptible to isolate Xv 82-8 of race 1 (Table 6-5 and 6-6) but wide

Table 6-4. Number of lesions per 2 cm^2 of leaf and diameter per lesion of parents, F_1, F_2, both backcrosses, and check lines two weeks after inoculation with 1.1 x 10^3 cfu ml^{-1} of isolate Xv 82-7 of race 2 of Xanthomonas campestris pv. vesicatoria.

Generation or cultivar	Numbers of plants	Lesions per 2 cm^2	Diameter per lesion (mm x 10)
Florida VR-4	8	5.4 ± 0.71[a]	1.99 ± 0.06
189-5	31	0	-
F_1 (Fla. VR-4 x 189-5)	39	0.42 ± 0.13	1.32 ± 0.18
F_2	282	1.95 ± 0.19	1.80 ± 0.07
F_1 x Fla. VR-4	44	2.94 ± 0.52	1.75 ± 0.11
F_1 x 189-5	47	0.10 ± 0.03	1.60 ± 0.41
Controls			
10 R	10	1.63 ± 0.38	1.17 ± 0.07
Early Calwonder	9	10.9 ± 1.55	5.8 ± 0.58

[a]Mean ± standard error of mean.

Table 6-5. Frequency distribution of number of lesions per 2 cm^2 of leaf in parental, F_1, F_2, and both backcross generations inoculated with race 1 isolate Xv 82-8 of Xanthomonas campestris pv. vesicatoria.

Generation	Lesions per 2 cm^{2a}										
	0 to <3	3 to <6	6 to <9	9 to <12	12 to <15	15 to <18	18 to <21	21 to <24	24 to <27	27 to <30	>30
	Number of plants										
Fla. VR-4							2	2	1	2	
189-5	16	7	4	2							
F_1 (Fla VR-4 x 189-5)			1	3	8	9	3	6	5	3	1
F_2	8	11	13	24	37	51	49	39	26	12	8
F_1 x Fla VR-4				1	3	2	12	13	8	4	1
F_1 x 189-5	6	3	7	11	3	5	2	4	4	2	

[a]Inoculum concentration 1.3×10^3 cfu ml^{-1}.

Table 6-6. Frequency distribution of diameter per lesion in parental, F_1, F_2, and both backcross generations inoculated with race 1 isolate Xv 82-8 of Xanthomonas campestris pv. vesicatoria.

Generation	Diameter per lesion (mm x 10)[a]								
	0 to <2	2 to <4	4 to <6	6 to <8	8 to <10	10 to <12	12 to <14	14 to <16	>16
	Number of plants								
Fla VR-4					1		2	2	2
189-5	19	8	2						
F_1 (Fla VR-4 x 189-5)		2	14	14	7	2			
F_2	5	49	77	61	39	19	9	7	14
F_1 x Fla VR-4			1	5	11	13	5	4	5
F_1 x 189-5	9	21	8	4	1	2	0	1	1

[a]Inoculum concentration 1.3×10^3 cfu ml^{-1}.

Table 6-7. Frequency distribution of number of lesions per 2 cm^2 of leaf in parental, F_1, F_2, and both backcross generations inoculated with race 3 isolate Xv 77-3A of Xanthomonas campestris pv. vesicatoria.

Generation	Lesions per 2 cm^{2a}										
	0 to <3	3 to <6	6 to <9	9 to <12	12 to <15	15 to <18	18 to <21	21 to <24	24 to <27	27 to <30	>30
	Number of plants										
Fla. VR-4					2		3	3			
189-5	30										
F_1 (Fla VR-4 x 189-5)	27	10	2								
F_2	152	31	21	16	24	15	7	10	1		2
F_1 x Fla VR-4	44	2	1								
F_1 x 189-5	17	4	2	4	5	6	2	1			

[a]Inoculum concentration 1.5 x 10^3 cfu ml^{-1}.

Table 6-8. Frequency distribution of diameter per lesion in parental, F_1, F_2, and both backcross generations inoculated with race 3 isolate Xv 77-3A of Xanthomonas campestris pv. vesicatoria.

Generation	Diameter per lesion (mm x 10)[a]				
	0 to <2	2 to <4	4 to <6	6 to <8	8 to <10
	Numbers of plants				
Fla. VR-4		2	3	2	1
189-5	30				
F_1 (Fla. VR-4 x 189-5)	11	20	7	1	
F_2	89	146	39	4	1
F_1 x Fla. VR-4	3	29	10	2	
F_1 x 189-5	36	10	1		

[a]Inoculum concentration 1.5 x 10^3 cfu ml^{-1}.

variation in both components of resistance, namely number of lesions per 2 cm^2 of leaf and diameter per lesion, occurred in the F_2 and backcross (F_1 x 189-5) populations. Values were continuous however and did not fit into any discreet segregation pattern or ratio. The distributions of lesion numbers and lesion diameters were skewed toward susceptibility, which suggests that resistance to race 1 is recessively inherited.

The F_1 and backcross (F_1 x 189-5) populations were resistant to race 3 isolate Xv 77-3A (Tables 6-7, 6-8, and 6-9), and wide variation occurred in the F_2 population. The F_2 frequency distributions for both components of resistance were continuous but strongly skewed toward the resistant parent (Tables 6-7 and 6-8). This strongly suggests that resistance to race 3 is dominant.

Two distinct groups of equal numbers of plants occurred in the backcross (F_1 x Fla. VR-4) population. One group was classified as resistant. This group had mean values of both components not significantly different from the F_1 population means (Table 6-9). The other group of backcross (F_1 x Fla. VR-4) plants was classified as susceptible (Table 6-9). The mean values of both components in this group were significantly greater ($P < 0.01$) than those of the resistant group in this population but slightly lower ($P < 0.05$) than in the susceptible parent Fla. VR-4. This strongly indicated that resistance to race 3 is controlled by a single dominant gene.

Despite continuous distribution of both components in the F_2 an attempt was made to classify plants into categories of either resistant or susceptible to isolate Xv 77-3A of race 3. This was achieved on the basis of observed phenotype, and evaluation of data of components of resistance. Plants with fewer than 8.7 lesions per

Table 6-9. Number of lesions per 2 cm^2 of leaf and diameter per lesion in resistant and susceptible plants in parental, F_1, F_2, both backcrosses, and check lines inoculated with 1.5×10^3 cfu ml^{-1} of race 3 isolate 77-3A of Xanthomonas campestris pv. vesicatoria.

Generation or cultivar	Resistant plants			Susceptible plants		
	Number	Lesions per 2 cm^2	Diameter[a] per lesion (mm x 10)	Number	Lesions per 2 cm^2	Diameter[a] per lesion (mm x 10)
Fla. VR-4	-	-	-	8	18.5 ± 1.23[b]	5.14 ± 0.72
189-5	30	0.13 ± 0.05	1.2 ± 0.21	-	-	-
F_1 (Fla. VR-4 x 189-5)	39	2.09 ± 0.32	2.94 ± 0.22	-	-	-
F_2	203	2.12 ± 0.16	2.33 ± 0.07	76	16.03 ± 0.60	3.83 ± 0.13
F_1 x Fla. VR-4	22	2.07 ± 0.31	2.68 ± 0.23	22	16.63 ± 0.98	3.79 ± 0.21
F_1 x 189-5	47	0.85 ± 0.18	1.79 ± 0.16	-	-	-
Controls						
10 R	-	-	-	10	12.4 ± 1.64[b]	3.68 ± 0.83
Early Calwonder	-	-	-	9	13.7 ± 0.92	3.59 ± 0.24

[a]Calculated from those plants with visible lesions.
[b]Mean ± standard error of mean.

2 cm^2 of leaf combined with lesions smaller than 0.30 mm were considered resistant. This classification resulted in 203 resistant F_2 plants, and 76 susceptible (Table 6-9). Difficulty was found in classifying 13 (4.7%) of these plants. However, this ratio is also consistent with a single dominant gene for resistance homozygous in 189-5 (chi^2 = 0.75, P = 0.5-0.3). It should be noted that mean values of both components did not differ significantly (P >0.05) among resistant plants in F_1, F_2, and backcross (F_1 x Fla. VR-4) populations (Table 6-9). These means also were marginally but significantly greater (P <0.05) than resistant plants in the 189-5 and backcross (F_1 x 189-5) populations.

Susceptibility to both isolates Xv 77-3A and Xv 82-8 was correlated. All F_2 plants susceptible to isolate Xv 77-3A of race 3 were, without exception, susceptible to isolate Xv 82-8 of race 1. Resistance to isolate Xv 82-8 occurred only among F_2 plants resistant to isolate Xv 77-3A. All plants of the backcross (F_1 x Fla. VR-4) were susceptible to isolate Xv 82-8 irrespective of reaction (resistant or susceptible) to isolate XV 77-3A. The diameters of lesions of both isolates were moderately correlated in F_2 plants (r=0.61, P <0.05), whereas numbers of lesions per 2 cm^2 of leaf were only weakly correlated (r=0.33).

Resistance to isolate Xv 77-3A of race 3 was dominant, under simple genetic control, but quantitative and correlated with quantitative resistance to isolate Xv 82-8 of race 1. Resistance to isolate Xv 82-8 was recessive. The same gene(s) could be responsible for both resistances. To investigate this further, analysis was made of weighted generation means of both components of resistance, namely lesion numbers per 2 cm^2 of leaf and lesion diameter. As expected, single gene models

fit the data for lesion numbers with both isolates (Tables 6-10 and 6-11). Dominance was incomplete for high lesion numbers with isolate Xv 82-8, and for low numbers with isolate Xv 77-3A. A single gene model adequately fitted generation means of lesion diameter with isolate Xv 77-3A but marginally failed to fit with isolate Xv 82-8 (Table 6-11). A digenic model with additive epistasis ('aa' in Table 6-10) fit the lesion diameter means with isolate Xv 82-8. Dominance for lesion diameter was minor with isolate Xv 77-3A, and negligible with isolate Xv 82-8; in both cases additivity was significant and was the parameter of major importance. Statistical deviation from a single gene model for lesion diameter with isolate Xv 82-8 was probably a result of sampling error. Means were based on few measurements on Florida VR-4. Generation means of lesion numbers and lesion diameters were correlated at $r=0.75$ and $r=0.94$ ($P < 0.05$), respectively, for isolates Xv 82-8 and Xv 77-3A.

Additive inheritance is based on a linear increase in means with increased frequency of the allele(s) controlling greater values (Falconer, 1960). Dominance is assessed as a deviation from linearity. Additivity was the major component of inheritance of lesion diameter, but reversal of dominance of lesion numbers occurred between the isolates. Dominance deviation for high lesion number was noted with the vigorous growing isolate Xv 82-8, and for low lesion number with the weak isolate Xv 77-3A.

These relationships are shown further in Figure 6-3 for lesion numbers, and Figure 6-4 for lesion diameter. The theoretical frequency of one allele of a single gene in F_1 and F_2 progenies is 0.5, and is 0.25 and 0.75 in respective backcrosses. Generation means are plotted

Table 6-10. Generation means analysis of number of lesions per 2 cm^2 of leaf and lesion diameter from Florida VR-4 x 189-5 progenies inoculated with isolate Xv 82-8 of race 1 of Xanthomonas campestris pv. vesicatoria.

	Population Means			
	Lesions per 2 cm^2		Diameter per lesion (mm x 10)	
Generation	Mean	Variance of mean	Mean[a]	Variance of mean
Fla. VR-4	23.00	2.0580	14.60	1.3024
189-5	3.51	0.2120	1.73	0.0091
F_1 (Fla. VR-4 x 189-5)	18.60	0.8602	6.51	0.0838
F_2	17.42	0.1628	6.51	0.1039
F_1 x Fla. VR-4	21.24	0.3977	11.70	0.5863
F_1 x 189-5	12.74	1.4010	4.66	0.3419
Parameter[b]	Estimate	S.E.[c] of estimate	Estimate	S.E.[c] of estimate
m	13.68*	0.561	6.89*	0.687
a	9.91*	0.562	6.72*	0.489
d	6.25*	1.122	-0.29	0.858
aa	-		1.56	0.856
Goodness of fit test	$X^2_{3df} = 6.11 < X^2_{p=0.95} = 7.82$		$X^2_{2df} = 5.64 < X^2_{p=0.95} = 5.99$	

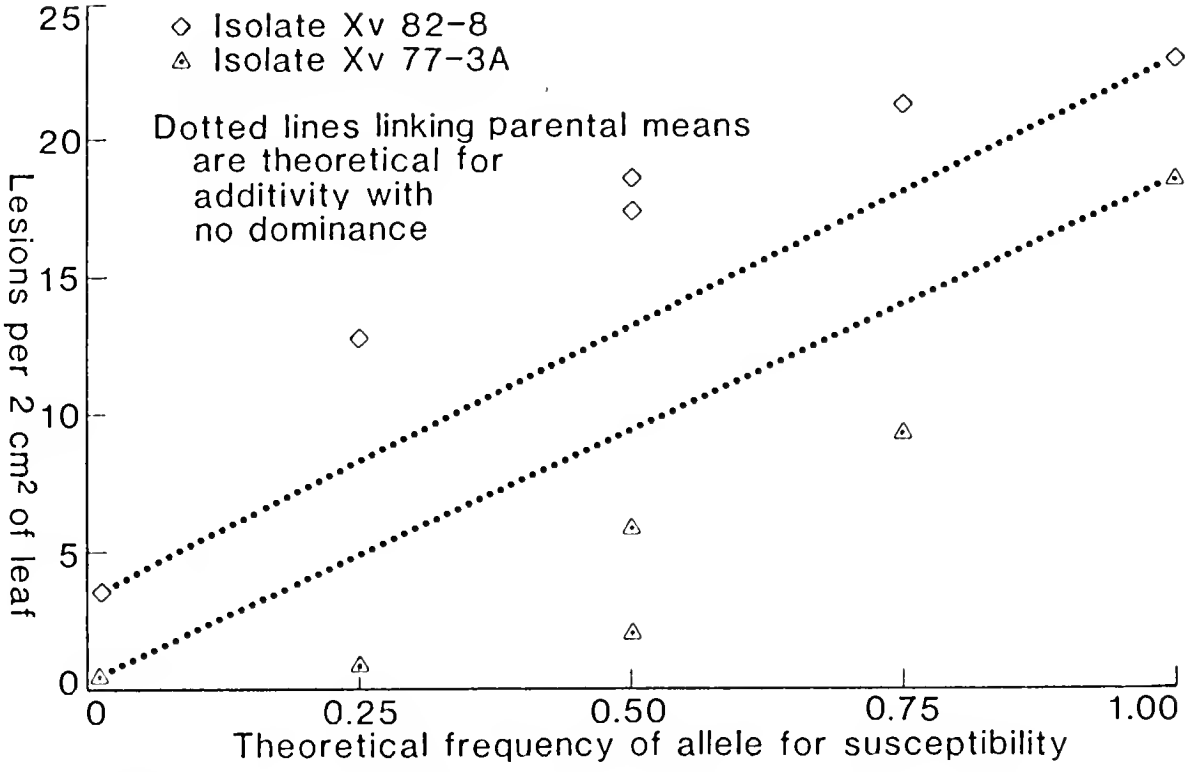
[a]Non-zero values only in means for lesion diameter.
[b]Parameters: m = estimated mean at F∞; a = additivity; d = dominance deviation; aa = additive epistasis.
[c]Standard error of parameter estimate.
*Significantly different from zero at P<0.05.

Table 6-11. Generation means analysis of number of lesions per 2 cm^2 of leaf and mean lesion diameter from Florida VR-4 x 189-5 progenies inoculated with isolate Xv 77-3A of race 3 of Xanthomonas campestris pv. vesicatoria.

	Population Means			
	Lesions per 2 cm^2		Diameter per lesion (mm x 10)	
Generation	Mean	Variance of mean	Mean[a]	Variance of mean
Fla. VR-4	18.50	1.162	5.14	0.394
189-5	0.13	0.003	1.04	0.002
F_1 (Fla. VR-4 x 189-5)	2.09	0.095	2.94	0.044
F_2	5.81	0.172	2.80	0.006
F_1 x Fla. VR-4	9.25	1.467	3.27	0.029
F_1 x 189-5	0.84	0.033	1.88	0.029
Parameter[b]	Estimate	S.E.[c] of estimate	Estimate	S.E.[c] of estimate
m	9.37*	0.428	2.62*	0.1523
a	9.27*	0.426	1.58*	0.1522
d	-7.73*	0.461	0.25	0.2580
Goodness of fit test	$X^2_{3df} = 3.64 < X^2_{p=0.95} = 7.82$		$X^2_{3df} = 5.48 < X^2_{p=0.95} = 7.82$	

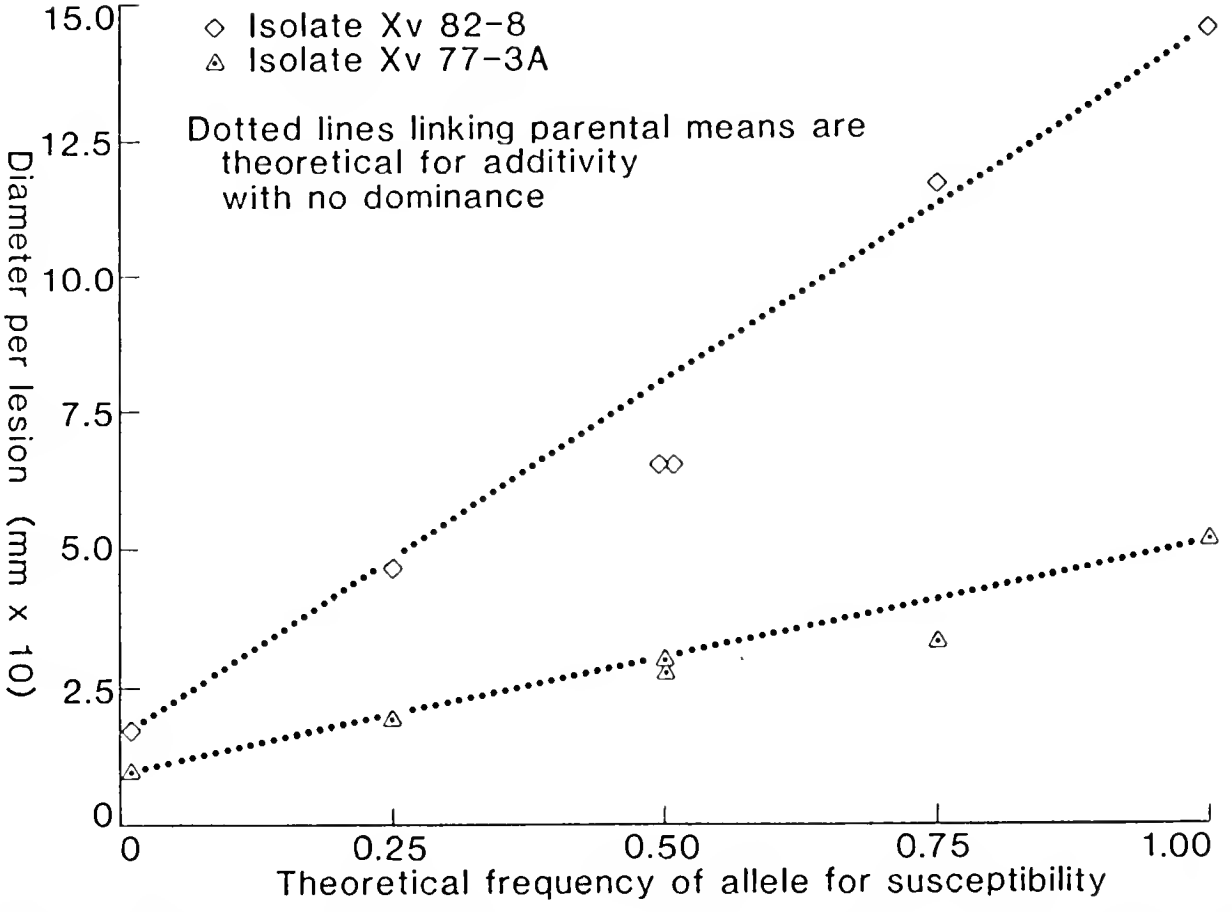
[a]Non-zero values only in means for lesion diameter.
[b]Parameters: m = estimated mean at F_∞; a = additivity; d = dominance deviation.
[c]Standard error of parameter estimate.
*Significantly different from zero at $P<0.05$.

Figure 6-3. Variation in number of lesions per 2 cm^2 of leaf related to theoretical frequency of the allele for susceptibility in pepper generations inoculated with two isolates of Xanthomonas campestris pv. vesicatoria.



Population tested	189-5 (resistant)	F_1 x 189-5	F_1 and F_2	F_1 x Fla. VR-4	Fla. VR-4 (susceptible)
Allele frequency	0	0.25	0.50	0.75	1.00

Figure 6-4. Variation in lesion diameters related to theoretical frequency of the allele for susceptibility in pepper generations inoculated with two isolates of Xanthomonas campestris pv. vesicatoria.



Population tested	189-5 (resistant)	F_1 x 189-5	F_1 and F_2	F_1 x Fla. VR-4	Fla. VR-4 (susceptible)
Allele frequency	0	0.25	0.50	0.75	1.00

against these allele frequencies. Lesion diameters with both isolates increased linearly (Figure 6-4), but lesion numbers increased nonlinearly in accordance with dominance mentioned above (Figure 6-3).

Additive gene action basically controlled lesion diameter with both bacterial isolates. This resistance controlled a reduction in the rate of lesion expansion (i.e. bacterial multiplication) in linear proportion with frequency of the resistance allele(s). Bacterial multiplication is so restricted in homozygous resistant plants (Tables 6-1 and 6-3, Figure 6-2) that very few necrotic areas become visible lesions. However, more necrotic areas grow to become visible lesions when resistant plants are inoculated with an aggressive isolate than with a weak growing isolate (Tables 6-5 and 6-6). The reduced rate of lesion expansion in heterozygotes compared with homozygous susceptible plants is not sufficient to prevent all potential lesions of an aggressive isolate from becoming visible, but is sufficient to prevent most necroses of weak isolates from becoming visible (Figures 6-3 and 6-4). Consequently, reversal of dominance of apparent gene action controlling lesion numbers will occur in proportion with the aggressiveness of the bacterial isolates.

Similar results to those reported here occurred with nonhypersensitive resistance in other PI lines (Chapters 3 and 7 of this dissertation). Stall (1981) also observed that apparently recessive gene action controlled lesion numbers, and also noted but did not quantify differences in lesion diameter among segregating plants.

The evidence presented led to the conclusion that the one and same gene in 189-5 controlled resistance to isolates of both races 1 and 3.

Plants of PI 163189 lacking the $\underline{Bs_1}$ gene were nevertheless resistant to race 2 (Table 6-1). The one additively inherited gene may be effective against all races of Xcv, and be responsible for durability of resistance. Quantitative variation observed among generations for numbers of hypersensitive flecks with isolate XV 82-7 of race 2 (Table 6-4) may reflect segregation of this gene.

Practical use of additive resistance in breeding is important. Resistance in heterozygotes varied with the aggressiveness of the isolate. It should be assumed that naturally occurring isolates of Xcv are aggressive. In this event, each cycle of selection for a high degree of resistance in recurrent backcrosses to bell pepper will require inbred progenies. Alternatively, susceptible but heterozygous parents may be identified by progeny testing in single generation cycles of recurrent backcrossing (Hanson, 1959). A third alternative involves identifying heterozygotes by inoculating with a weak growing isolate (see also this dissertation, Chapter 3).

Reference to many recessive resistances to bacterial plant pathogens, including pepper, occur in the literature. For example, Brinkerhoff et al. (1984), Chand and Walker (1964a and b), Fallik et al (1984), Hibberd and Gillespie (1982a), Innes et al. (1984), Patel (1982), and Stall (1981), have reported them. In some, the resistance was incompletely recessive in heterozygotes (Hibberd and Gillespie, 1982a). Sidhu and Khush (1977) reported reversal of dominance in heterozygotes may occur with aging of plants.

Inheritance of the two resistance genes occurring in selection 189-5 of PI 163189 appears fundamentally different. The dominant gene

$\underline{Bs_1}$ was epistatic to additively inherited resistance. Nevertheless, change in bacterial populations *in vivo* of all three races (Figure 6-2) were consistent with hypersensitivity (Klement et al., 1964). Further comparative study of these resistances is justified.

CHAPTER 7
INHERITANCE OF NONHYPERSENSITIVE RESISTANCE TO BACTERIAL SPOT IN A PLANT INTRODUCTION OF PEPPER (*Capsicum annuum* L.)

Resistance to bacterial leaf spot incited by *Xanthomonas campestris* pv. *vesicatoria* (Doidge, 1920), Dye 1978 occurs in numerous plant introductions (PI) of pepper (Dempsey, 1953; Greenleaf 1960; Kim, 1983; Sowell, 1960; Sowell and Dempsey, 1977; Sowell and Langford, 1963). Resistance in many of these lines has proven effective and durable in diverse environments (Adamson and Sowell, 1983; Borchers, 1965; Hibberd and Gillespie, 1982a; Hibberd et al., 1979; Kim and Hartmann, 1979; Stall, 1981; Chapter 8, this dissertation). Several lines were heterogeneous for race specific resistance controlled by dominant genes for hypersensitivity (Cook and Stall, 1969; Kim and Hartmann, 1985; Chapters 3 and 6, this dissertation), but most lacked these genes (Cook and Stall, 1969).

Releases of bell pepper have been made which contain genes for hypersensitivity (Cook, 1984; A.A. Cook, 1979 and 1982, personal communications). Releases incorporating nonhypersensitive resistance have not been made. That is because of the difficulty of identification of resistant segregates (Adamson and Sowell, 1983; Hibberd and Gillespie, 1982a), and the common assumption that resistance is controlled by multiple genes (Cook and Stall, 1969).

It is important that genes controlling durable resistance be identified. Simultaneously, efficient selection tests need to be developed to make resistance more accessible to plant breeders. I chose to evaluate nonhypersensitive resistance in a PI line known to be durably resistant (Greenleaf, 1960; Chapter 8, this dissertation).

Materials and Methods

Seed Sources

Seed of the PI 246331 was obtained from the USDA South Atlantic Region Plant Introduction Station, Georgia. Seed of other peppers were on hand: Early Calwonder (ECW), line 271-4 which has genes $\underline{Bs_1}$ and $\underline{Bs_3}$ for hypersensitivity to races 2 and 1, respectively (Chapter 3, this dissertation), and 10 R which is near isogenic with ECW and having gene $\underline{Bs_1}$ (Dahlbeck et al., 1979).

Inoculum Preparation

The bacterial cultures and their race designation used in this study were isolates Xv 0623, Xv 77-3, and Xv 82-8 of race 1, Xv 82-7 and Xv 83-1 of race 2, and Xv 77-3A of race 3. These were from the culture collection of R. E. Stall. Isolate Xv 77-3 was recovered from lyophilized storage and the others were kept in refrigerated sterile water. Isolate Xv 77-3A (race 3) was a mutant form of Xv 77-3 (race 1). Inocula were prepared from agitated (24 h) nutrient broth cultures. After centrifugation, bacterial pellets were resuspended in sterile tap water, and standardized colorimetrically to 50% light transmittance. These suspensions approximate a density of 5×10^8 cfu (colony forming units) ml^{-1} and were either used directly, or were serially diluted to final concentrations of 1×10^3 to 3×10^3 cfu ml^{-1}. Final concentrations were confirmed by replicated colony counts from 0.05 ml subsamples spread on nutrient agar plates. Correct race designation was confirmed by the different reactions to inoculation with 5×10^8 cfu ml^{-1} of peppers ECW, 10 R, and 271-4 (Chapter 4, this dissertation). All inoculations were by hypodermic infiltration of intercostal leaf tissues.

Heterogeneity of Resistance in PI 246331

An estimate of the variability in resistance of PI 246331 to three races of Xcv was obtained from a small population of plants. Plants of PI 246331, ECW, and 10 R were cultured in steamed, peat-vermiculite mix in 10-cm plastic pots arranged in a greenhouse (temperature range 20 to 35 C). Rows of eight plants were randomized. Plants were watered as required and treated four times during the experiment with approximately 0.4 g per pot of soluble 20:20:20 fertilizer. Four fully expanded leaves per plant of PI 246331 and ECW were each inoculated with approximately 2.5×10^3 cfu ml^{-1} of each of the four isolates Xv 0623 and Xv 82-8 (race 1), Xv 82-7 (race 2), and Xv 77-3A (race 3). Spots about 2 cm diameter of each leaf were infiltrated with inoculum of all four isolates. A single leaf was collected from each plant at 11, 14, 21, and 32 days following inoculation. The number of lesions per 2 cm^2 of leaf within a perimeter imprinted by a cork-borer was counted at each inoculation site viewed under a dissecting microscope (magnification 2.5 X). The diameters of five randomly chosen lesions at each site (or of all lesions where fewer than five existed) were measured at day 32 using a graduated eyepiece. The mean values were obtained for each plant and isolate combination.

In addition to the above test, all plants of PI 246331, ECW, and 10 R were observed for hypersensitive reactions (HR) which may develop within 24 h after inoculating two additional leaves per plant with 5×10^8 cfu ml^{-1} of each isolate. A single plant selection, designed 246-4 was made for a high degree of resistance to all isolates. Inbred progeny of 246-4 were used in subsequent experiments.

Bacterial Populations in vivo

Low disease severity in plants of 246-4 was expected to correlate with low populations of bacteria in mesophyll in comparison with susceptible plants. Plants of 246-4, ECW, and 10 R were raised in a greenhouse as described above. Three fully expanded leaves per plant were each inoculated with three isolates each at approximately 1.5×10^3 cfu ml^{-1}. The isolates were Xv 80-5 (race 1), Xv E3 (race 2), and Xv 69-1 (race 3). Single plants of each host were collected periodically after inoculation, that is, at 0, 2, 5, 8, 11, and 14 days. Populations of bacteria of each race were determined from triplicated 1.0 cm^2 (i.e., 2×0.5 cm^2) leaf samples. Samples were triturated in 0.5 ml sterile water, the suspensions serially diluted where appropriate, and 0.05 ml subsamples of the final dilutions spread on nutrient agar plates. Colonies were counted after 2 to 3 days incubation at 30 C and mean values converted to $\log_{10}$ (cfu cm^{-2}) of leaf. The numbers of lesions per 2 cm^2 of leaf and diameters of a maximum of five lesions per inoculation site were obtained on additional plants 15 days after inoculation. The experiment was repeated.

Inheritance of Resistance

A single plant of ECW was crossed with pollen from a single inbred progeny plant from 246-4. A single F_1 plant was self-pollinated to yield F_2 seed. This F_1 plant and additional plants of respective parents were cross-pollinated to give backcross progenies.

Plants of populations of parents, F_1, F_2, and backcrosses were raised in a greenhouse in randomized rows of eight plants as described above. When plants had grown to the stage of the first fork of the main stem, the following inoculations were applied to all plants. Three fully expanded leaves below the first fork were inoculated with each of the isolates Xv 82-8 (race 1), Xv 82-7 (race 2), and Xv 73-3A (race 3). Inoculum concentration of each was approximately 1.5×10^3 cfu ml^{-1}. Each leaf was inoculated with all three isolates. Inoculated leaves were harvested after 2.5 weeks, sealed in plastic bags and refrigerated (3 C). Over several days lesions were counted and measured as described above, and mean values per plant were obtained for each isolate.

The same plants were inoculated a second time 3 weeks later to confirm the inheritance of resistance. Two leaves at the main fork of the stem of each plant were each inoculated with isolates Xv 77-3 (race 1) and Xv 83-1 (race 2). Leaves were harvested after 2.5 weeks. Lesions were counted and measured as described above. Generation means weighted by their variances were analyzed to determine gene action (Basford and De Lacy, 1979) controlling both number of lesions per 2 cm^2 of leaf and diameter per lesion with each isolate.

Results and Discussion

Heterogeneity of Resistance in PI 246331

All inoculated plants of PI 246331 were resistant to four isolates of Xcv comprising races 1, 2, and 3. Relatively few lesions per 2 cm^2 of leaf developed on plants of PI 246331 infiltrated with approximately 2.5×10^3 cfu ml^{-1} (Table 7-1), but variation occurred among plants.

Table 7-1. Number of lesions per 2 cm^2 of leaf at timed intervals and diameter per lesion at 32 days after inoculation of peppers Early Calwonder and PI 246331 with four isolates of *Xanthomonas campestris* pv. *vesicatoria*.

Xcv isolate			Lesions per 2 cm^2 of leaf				Diameter per lesion at
			Days after inoculation[a]				
Identity	Race	Host	11	14	20	32	32 days (mm)
Xv 0623	1	PI 246331	6.2 ± 2.98[b]	8.9 ± 3.98	5.4 ± 2.33	9.5 ± 3.66	0.20 ± 0.05
		ECW[c]	7.8 ± 2.13	19.7 ± 2.48	33.5 ± 4.15	41.5 ± 5.59	0.80 ± 0.04
Xv 82-8	1	PI 246331	6.6 ± 2.07	10.4 ± 3.04	8.6 ± 2.44	12.0 ± 1.52	0.19 ± 0.04
		ECW	8.2 ± 1.19	10.8 ± 2.24	18.6 ± 2.93	23.7 ± 4.08	0.70 ± 0.02
Xv 82-7	2	PI 246331	3.5 ± 2.41	6.0 ± 1.47	6.3 ± 3.36	11.6 ± 5.58	0.24 ± 0.08
		ECW	3.3 ± 1.28	6.7 ± 0.82	13.5 ± 2.69	18.0 ± 7.18	1.00 ± 0.05
Xv 77-3A	3	PI 246331	6.5 ± 3.69	8.1 ± 3.31	12.0 ± 4.76	14.2 ± 6.78	0.13 ± 0.05
		ECW	10.2 ± 3.40	19.6 ± 3.91	26.8 ± 5.04	52.8 ± 11.50	0.70 ± 0.06
General mean		PI 246331	5.7	8.4	8.1	11.8	0.19
		ECW	7.4	13.2	23.1	34.0	0.80

[a]Inoculum concentration approximately 2.5×10^3 cfu ml^{-1} for each isolate.
[b]Mean ± standard error of mean.
[c]ECW = Early Calwonder.

This contrasted with relatively many lesions in ECW. Lesion diameter 32 days after inoculation was approximately four times as great in ECW as in PI 246331, but variation occurred among plants of PI 246331 in this attribute also. One plant, designated 246-4, was selected for very few lesions of small size with all isolates. Inbred progeny of 246-4 were homozygous and homogeneous for their reaction to these same isolates.

Plants of 10 R were uniformly hypersensitive within 24 h after inoculation with 5 x 10^8 cfu ml^{-1} of isolate Xv 82-7 of race 2. A susceptible reaction occurred in leaves of 10 R inoculated with isolates of races 1 and 3, and in ECW with races 1, 2, and 3. The susceptible reaction was characterized by watersoaked appearance of the tissue 24 to 36 h after inoculation. This was followed by tissue necrosis in 2.5 to 3 days. The necrosis that occurred in plants of PI 246331 differed from both the HR and the susceptible reaction. This necrosis developed in 2 to 3 days after inoculation, was dry and brown, and was not preceded by a watersoaked appearance. Necrosis observed by Cook and Stall (1969) in plants of a larger population of PI 246331 was interpreted as indistinguishable from the susceptible reaction. However, plants in the present test were clearly resistant (Table 7-1). Data from field tests support the resistance reaction (Chapter 8, this dissertation). Interpretation of the resistance reaction in PI 246331 to inoculation with high concentrations of bacteria should depend on observations of ultrastructure and physiology (Jones and Fett, 1985).

Bacterial Populations in vivo

Populations of bacteria in leaves of ECW multiplied to 10^7 to 10^8 cfu cm^{-2} of leaf in 12 to 14 days after inoculation with low

concentrations of bacteria (Table 7-2). In contrast, bacteria in leaves of selection 246-4 multiplied for only a few days and reached maximum recorded values that were about 10^3 to 10^4 fewer than in leaves of ECW. Bacterial populations in leaves of 246-4 slowly declined after 5 days. This pattern of change in bacterial populations is consistent with, and difficult to distinguish from that expected with hypersensitivity (Cook and Guevara, 1984; Klement et al., 1964; Stall and Cook, 1966).

Few lesions per unit area of leaf occurred with any bacterial isolate in plants of 246-4 in this experiment (Table 7-3). This contrasted with relatively many lesions of large diameter in leaves of ECW. Diameters of the few lesions in 246-4 were much smaller than in ECW ($P<0.01$), but several lesions were of unexpectedly greater diameter and appeared as raised pustules. Nevertheless, low disease severity in 246-4 accurately reflected relatively low populations of bacteria in leaves.

Inheritance of Resistance

Differences were noted among the isolates Xv 82-8, Xv 82-7, and Xv 77-3A in lesion diameter in all generations of plants used to study inheritance of resistance. Lesion diameters were greatest with isolate Xv 82-8, and least with isolate Xv 77-3A, and the differences were consistent across generations (Table 7-4). These differences are thought not to reflect the effect of race, but simply the aggressiveness of each isolate (see also Chapters 3 and 6 of this dissertation). Differences occurred among the isolates in the numbers of lesions of 2 cm^2 of leaf but this is taken to reflect different inoculum concentrations (Table 7-4).

Table 7-2. Estimated bacterial populations per cm^2 of leaf in samples from peppers 246-4 and Early Calwonder inoculated with races 1, 2 and 3 of Xanthomonas campestris pv. vesicatoria.

Bacterial culture[a]	Host line	Log_{10} of numbers of bacteria per cm^2 of leaf: Days after inoculation 0	2	5	8	11	14
Xv 80-5	246-4	0.98	2.69[b]	3.04	3.14	3.52	1.77
Race 1	ECW[c]	0.98	2.65	6.16	6.83	7.52	7.99
Xv E3	246-4	1.18	2.00	4.53	1.84	3.54	1.06
Race 2	ECW	1.18	3.69	6.49	7.35	7.30	7.76
Xv 69-1	246-4	0.75	1.90	3.36	2.20	0.48	1.64
Race 3	ECW	0.75	2.73	5.69	6.70	6.93	7.73

[a]Inoculum concentrations of races 1, 2, and 3 were, respectively, 1.4×10^3, 2.2×10^3 and 0.8×10^3 cfu ml^{-1}.
[b]Values are means of 6 replicates.
[c]ECW = Early Calwonder.

Table 7-3. Lesions per 2 cm^2 of leaf and diameter per lesion in peppers Early Calwonder and 246-4 15 days after inoculation with races 1, 2, and 3 of *Xanthomonas campestris* pv. *vesicatoria*.

Bacterial culture[a]		Host			
		Early Calwonder		246-4	
Designation	Race	Lesions per 2 cm^2	Diameter per lesion (mm x 10)	Lesions per 2 cm^2	Diameter per lesion (mm x 10)
Xv 80-5	1	24.4 ± 1.32[b]	7.0 ± 0.43	0.5 ± 0.23	2.9 ± 0.57
Xv E3	2	26.3 ± 1.56	7.3 ± 0.50	0.6 ± 0.23	3.0 ± 0.47
Xv 69-1	3	16.0 ± 1.23	6.3 ± 0.55	0.2 ± 0.08	2.4 ± 0.36

[a]Inoculum concentrations of races 1, 2, and 3 were respectively, 1.4×10^3, 2.2×10^3, and 0.8×10^3 cfu ml^{-1}.
[b]Mean ± standard error of mean.

Table 7-4. Number of lesions per 2 cm^2 of leaf and diameter per lesion in leaves of parental, F_1, F_2, and backcross populations of the cross between peppers Early Calwonder and 246-4 inoculated with races 1, 2, and 3 of Xanthomonas campestris pv. vesicatoria.

		Bacterial isolate[a]					
		Xv 77-3A Race 3		Xv 82-7 Race 2		Xv 82-8 Race 1	
Generation	Number of plants	Lesions per 2 cm^2	Diameter per lesion (mm x 10)	Lesions per 2 cm^2	Diameter per lesion (mm x 10)	Lesions per 2 cm^2	Diameter per lesion (mm x 10
ECW[b]	46	21.8 ± 0.77[c]	5.5 ± 0.24	18.6 ± 1.00	6.2 ± 0.25	38.9 ± 0.93	10.1 ± 0.42
246-4	56	0.1 ± 0.03	1.9 ± 0.45	0.1 ± 0.03	2.0 ± 0.28	13.0 ± 0.49	2.1 ± 0.07
F_1 (ECW x 246-4)	46	7.2 ± 0.61	2.8 ± 0.10	5.1 ± 0.44	3.7 ± 0.12	42.3 ± 1.15	5.6 ± 0.13
F_2	280	6.1 ± 0.37	2.8 ± 0.10	3.5 ± 0.25	3.3 ± 0.08	38.3 ± 0.53	6.2 ± 0.23
F_1 x ECW	186	16.9 ± 0.51	4.5 ± 0.10	12.1 ± 0.45	5.3 ± 0.10	41.5 ± 0.48	8.0 ± 0.18
F_1 x 246-4	167	1.7 ± 0.45	1.9 ± 0.07	0.5 ± 0.08	1.9 ± 0.12	30.3 ± 0.82	3.9 ± 0.11

[a]Inoculum concentrations were approximately 1.6 x 10^3, 1.3 x 10^3 and 2.8 x 10^3 for, respectively, isolates Xv 77-3A, Xv 82-7, and Xv 82-8.
[b]ECW = Early Calwonder.
[c]Mean ± standard error of mean.

Significant variation occurred among generation means for numbers of lesions per 2 cm^2 of leaf with each of the bacterial isolates (Table 7-4). Relatively many lesions occurred in leaves of ECW and few lesions occurred in 246-4 with all isolates. However, many more lesions of isolate Xv 82-8 occurred in leaves of 246-4 than of isolates Xv 77-3A and Xv 82-7, despite the different inoculum concentrations. Plants of 246-4 appeared less resistant to the aggressive isolate Xv 82-8 than to the other two isolates which grew less vigorously in this experiment.

The mean number of lesions per 2 cm^2 of leaf with isolates Xv 77-3A and Xv 82-7 in F_1 and segregating populations fell between the values recorded for both parents but were skewed toward the resistant parent (Table 7-4). Means for the F_1 and F_2 populations with both isolates were lower than the mid-parent value, and the mean of the backcross (F_1 x ECW) population approximated closely the means of the the F_1 and ECW populations. These data are interpreted to mean that incomplete dominance occurred along with additive gene action to control low numbers of lesions with these isolates. In contrast, high numbers of lesions occurred with isolate Xv 82-8 (Table 7-4). The means of the ECW, F_1, F_2, and backcross (F_1 x ECW) populations were approximately equal with this isolate, and the mean of the backcross (F_1 x 246-4) population closely approximated the average of the F_1 and 246-4 means. Thus, dominant gene action controlled high lesion numbers with this isolate.

The frequency distributions of lesion numbers for all three isolates in these populations were continuous (Tables 7-5 and 7-6), and distinct classes of resistant and susceptible plants did not occur. Consequently, the fitting of segregation ratios was not possible. Wide variation is expected to occur in the F_2 and the backcross to the susceptible parent for quantitative attributes controlled by simply inherited dominant gene action, and in the F_2 and backcross to the resistant parent if controlled by recessive gene action (Mather and Jinks, 1971, pages 127 to 137). Wide variation occurred for numbers of lesions in the F_2 and backcross (F_1 x ECW) with isolates Xv 77-3A and Xv 82-7, and in the F_2 and backcross (F_1 x 246-4) with isolate Xv 82-8, indicating that recessive and dominant gene action, respectively, are involved.

Two different genetic resistance mechanisms controlling numbers of lesions are implied by these data. This was examined further by goodness for fit of weighted generation means to simple additive-dominance, or digenic models of gene action (Basford and De Lacy, 1979). The computed genetic parameters of additivity, dominance, and epistasis were similar for both isolates Xv 77-3A and Xv 82-7, and only those of isolate Xv 77-3A are presented for comparison with isolate Xv 82-8.

A simple additive-dominance gene action model failed to fit the weighted generation means of lesion numbers of isolate Xv 77-3A (Table 7-7), and the full digenic model was necessary. Estimates of additivity, dominance, additive epistasis, and digenic dominance interactions all differed significantly from zero. However, this statistical evaluation of gene action is inappropriate for lesion numbers with this isolate since the parameter estimating the general population mean did not differ significantly from zero, or no disease. In contrast to this,

Table 7-5. Frequency distribution of lesions per 2 cm^2 of leaf in parents Early Calwonder and 246-4, and F_1, F_2 and backcross populations inoculated with race 1 of Xanthomonas campestris pv. vesicatoria.

Generation	Lesions[a] per 2 cm^2 of leaf											
	0 to <6	6 to <12	12 to <18	18 to <24	24 to <30	30 to <36	36 to <42	42 to <48	48 to <54	54 to <60	60 to <66	>66
	Numbers of plants											
ECW[b]					6	5	16	15	3			
246-4	7	21	13	12	2	1						
F_1 (ECW x 246-4)					2	5	17	12	8		2	
F_2			2	11	27	74	91	47	19	7	4	3
F_1 x ECW				1	4	32	61	58	25	6		
F_1 x 246-4		8	12	22	41	31	25	17	5	2		

[a]Inoculum concentration of isolate Xv 82-8 of race 1 was approximately 2.8×10^3 cfu ml^{-1}.
[b]ECW = Early Calwonder.

Table 7-6. Frequency distribution of lesions per 2 cm^2 of leaf in parents Early Calwonder and 246-4, and F_1, F_2, and backcross populations inoculated with races 2 and 3 of Xanthomonas campestris pv. vesicatoria.

Generation	Bacterial isolate	Lesions per 2 cm^2 of leaf: Number of plants											
		0	0 to <3	3 to <6	6 to <9	9 to <12	12 to <15	15 to <18	18 to <21	21 to <24	24 to <27	27 to <30	>30
ECW[b]	Xv 77-3A						2	5	11	11	12	3	2
	Xv 82-7				2	6	10	7	4	4	7	5	1
246-4	Xv 77-3A	46	10										
	Xv 82-7	50	6										
F_1 (ECW x 246-4)	Xv 77-3A	1	6	10	11	12	5	1					
	Xv 82-7	1	8	22	10	2	1	1					
F_2	Xv 77-3A	20	89	62	36	31	15	8	7	5	4	1	1
	Xv 82-7	44	120	64	19	15	7	4	3	1			
F_1 x ECW	Xv 77-3A			8	12	24	31	35	20	24	18	7	7
	Xv 82-7		9	23	34	41	21	20	16	15	6	1	2
F_1 x 246-4	Xv 77-3A	58	85	15	7	2							
	Xv 82-7	98	62	6	1								

[a]Inoculum concentrations were approximately 1.6 x 10^3 and 1.3 x 10^3 cfu ml^{-1} for isolates Xv 77-3A (race 3), and Xv 82-7 (race 2), respectively.
[b]ECW = Early Calwonder.

Table 7-7. Analysis of weighted generation means of number of lesions per 2 cm^2 of leaf and diameter per lesion in progenies of peppers Early Calwonder and 246-4 inoculated with isolate Xv 77-3A of *Xanthomonas campestris* pv. *vesicatoria*.

	Lesions per 2 cm^2 of leaf		Diameter per lesion (mm x 10)	
Parameter[a]	Estimate	S.E.[b] of estimate	Estimate	S.E. of estimate
m	-2.0	2.02	2.1*	0.38
a	10.8*	0.37	1.8*	0.18
d	23.3*	5.14	2.0	1.01
aa	12.9*	1.99	1.6*	0.33
ad	8.7*	1.54	1.4*	0.43
dd	-14.1*	3.37	-1.4*	0.67
Goodness of fit test	Not applicable[c]		Not applicable	

[a]Parameters: *m* = theoretical population mean at F_∞; *a* = additivity; *d* = dominance deviation; *aa* = additive epistasis; *ad* = additive dominance epistasis; *dd* = dominance epistasis.
[b]S.E. = standard error.
[c]All degrees of freedom used in parameter estimation.
*Significantly different from zero at $P<0.05$.

a digenic model with significant estimates of additivity, dominance, and additive epistasis (for slightly fewer lesions than expected with additivity only) fitted generation means of lesion numbers for isolate Xv 82-8 (Table 7-8). These data reliably reflected the earlier observation of a high degree of dominance for many lesions with this isolate.

Reversal of dominance of the genetic control of lesion numbers appeared to occur between the two isolates Xv 77-3A and Xv 82-8. However, these data did not take into account the large differences in lesion diameter occurring among the isolates (Table 7-4). These differences may influence interpretation of the inheritance of resistance, that is, more lesions are always produced by the most aggressive isolate (Chapters 3 and 6 of this dissertation).

Lesions of all isolates were of relatively large diameter in plants of ECW, and small in plants of 246-4 (Table 7-4). The generation means of lesion diameters of isolates Xv 77-3A and Xv 82-7 closely paralleled the pattern observed for lesion numbers with these isolates, that is, the means of the F_1 and segregating populations were between the parental means but skewed toward the mean of 246-4, and the mean of the backcross (F_1 x ECW) approximated the average of the F_1 and ECW populations. In contrast to this, the generation means for isolate Xv 82-8 clearly reflected additive gene action. The mean lesion diameters of the F_1 and F_2 populations closely approximated the mid-parent value, and the means of the two backcrosses were very close to the averages of the F_1 and respective parental means.

The frequency distributions of lesion diameters in these populations were continuous (Tables 7-9 and 7-10). It was not possible to

Table 7-8. Analysis of weighted generation means of numbers of lesions per 2 cm^2 of leaf and diameter per lesion in progenies of peppers Early Calwonder and 246-4 inoculated with isolate Xv 82-8 of *Xanthomonas campestris* pv. *vesicatoria*.

	Lesions per 2 cm^2 of leaf		Diameter per lesion (mm x 10)	
Parameter[a]	Estimate	S.E.[b] of estimate	Estimate	S.E. of estimate
m	34.4*	1.54	6.2*	0.14
a	12.3*	0.50	4.1*	0.14
d	7.4	7.50	-0.5*	0.21
aa	-8.6*	1.65		
Goodness of fit	$X^2_{2df} = 3.53 < X^2_{0.95} = 5.99$		$X^2_{3df} = 2.08 < X^2_{0.95} = 7.82$	

[a]Parameters: *m* = theoretical population mean at F_∞; *a* = additivity; *d* = dominance deviation; *aa* = additive epistasis.
[b]S.E. = standard error.
*Significantly different from zero at $P<0.05$.

Table 7-9. Frequency distribution of lesion diameter in parents Early Calwonder and 246-4, and F_1, F_2, and backcross populations inoculated with race 1 and Xanthomonas campestris pv. vesicatoria.

Generation	Diameter per lesion[a] (mm x 10) Number of plants											
	1 to <2	2 to <3	3 to <4	4 to <5	5 to <6	6 to <7	7 to <8	8 to <9	9 to <10	10 to <11	11 to <12	>12
ECW[b]					1	3	8	5	6	7	10	6
246-4	22	31	3									
F_1 (ECW x 246-4)			1	9	22	10	4					
F_2		5	30	50	62	66	35	25	6	3	3	
F_1 x ECW				8	27	36	33	23	21	16	10	12
F_1 x 246-4	5	48	37	36	22	9	5	1				

[a]Inocolum concentration of isolate Xv 82-8 of race 1 was approximately 2.8×10^3 cfu ml^{-1}.
[b]ECW = Early Calwonder.

Table 7-10. Frequency distribution of lesion diameter in parents Early Calwonder and 246-4, F_1, F_2, and backcross populations inoculated with races 2 and 3 of Xanthomonas campestris pv. vesicatoria.

Generation	Bacterial isolate	Diameter per lesion[a] (mm x 10)									
		0	>0 to <1	1 to <2	2 to <3	3 to <4	4 to <5	5 to <6	6 to <7	7 to <8	8 to <9	>9
		Number of plants										
ECW[b]	Xv 77-3A				1	9	7	12	7	8		2
	Xv 82-7					3	10	7	13	7	2	4
246-4	Xv 77-3A	46		5	4		1					
	Xv 82-7	50		1	4	1						
F_1 (ECW x 246-4)	Xv 77-3A	1		3	26	13	2					
	Xv 82-7	1		1	9	15	16	3				
F_2	Xv 77-3A	20		35	128	69	18	8	1			
	Xv 82-7	44		22	71	67	46	18	9			
F_1 x ECW	Xv 77-3A				23	54	53	34	12	5	4	1
	Xv 82-7				4	24	54	57	24	17	5	1
F_1 x 246-4	Xv 77-3A	58		50	51	5	3					
	Xv 82-7	98		33	26	5	3	1	1			

[a]Inoculation concentrations of approximately 1.6×10^3 and 1.3×10^3 cfu ml^{-1} for isolates Xv 77-3A (race 3) and Xv 82-7 (race 2), respectively.
[b]ECW = Early Calwonder.

define distinct segregation ratios for lesion diameter in segregating populations. Additivity and additive espistasis for large lesion diameters were significant in the analysis of weighted generation means with isolate Xv 77-3A (Table 7-7). Dominance and dominance epistasis were nonsignificant. In contrast, additivity and slight dominance for small lesions were significant for isolate Xv 82-8 (Table 7-8). With both isolates, additivity was the genetic parameter which most strongly reflected observed generation means of lesion diameter. Reversal of dominance that was observed for the lesion numbers did not occur with lesion diameters.

Resistance to disease induced by one isolate in F_2 plants was positively correlated with resistance to the remaining two isolates. The simple correlation in the F_2 between numbers of lesions caused by isolates Xv 77-3A and Xv 82-7 was $r = 0.61$. However, the same correlation between Xv 77-3A and Xv 82-8 was only $r = 0.25$. This low value is consistent with the apparent reversal of dominance for lesion numbers (Table 7-4). Correlations in the F_2 between lesion diameters of all three isolates were intermediate, that is, $r = 0.44$ for isolates Xv 82-7 and Xv 82-8, $r = 0.50$ for Xv 77-3A and Xv 82-7, and $r = 0.60$ for Xv 77-3A and Xv 82-8. The generation means of lesion diameters (Table 7-4) were highly correlated between isolates (r values between 0.96 and 0.99). Resistance to all three isolates was considered either linked or under the control of the same genetic mechanism.

Correlated resistance to all three isolates was examined further. Plants of the F_2 and backcross (F_1 x ECW) populations were noted that had numbers of lesions per 2 cm^2 of leaf of isolate Xv 77-3A greater than or equal to the population mean plus one standard deviation. The

means of both components of resistance for all three isolates were then computed for plants in the group of selected plants, and in the remaining plants. Comparisons of group means for each isolate were made by t-test (Table 7-11). For computations involving lesion diameter data, plants which had no lesions were excluded from the means. The numbers of lesions and diameter per lesion for all three isolates were significantly greater ($P<0.01$) in the group of F_2 plants selected only for high numbers of lesions of isolate Xv 77-3A then the remaining F_2 plants. Similarly, mean values of both components with isolates Xv 77-3A and Xv 82-7 were significantly greater in backcross (F_1 x ECW) plants noted for high numbers of lesions of isolate Xv 77-3A than in the remaining plants of that backcross. However, with isolate Xv 82-8, the difference in lesion numbers was not significant, and in lesion diameter was marginally nonsignificant ($P>0.05$) (Table 7-11).

These data support the hypothesis that a single genetic mechanism occurs in 246-4 to control resistance to all three races, but that the degree of resistance varied among isolates in accordance with their vigor of growth. Greater resistance occurred in segregating populations with the weaker growing isolates Xv 77-3A and Xv 82-7 than with isolate Xv 82-8.

Additive gene action which was important in controlling lesion diameter contrasted a high degree of dominance, either positive or negative, in the genetic control of low numbers of lesions (Table 7-4). In a bid to resolve this contrast, further data were collected on the same plants inoculated with low concentrations of two additional isolates, namely Xv 77-3 of race 1 and Xv 83-1 of race 2. Generation means of both components of resistance are presented in Tables 7-12 and 7-13 for isolates Xv 77-3 and Xv 83-1, respectively.

Table 7-11. Comparison of means of lesions per 2 cm^2 of leaf and diameter per lesion for each of three isolates of Xanthomonas campestris pv. vesicatoria among plants of the F_2 and first backcross to the susceptible parent selected only for high numbers of lesions of one of the isolates.

Selected plants	Bacterial isolate					
	Xv 77-3A Race 3		Xv 82-7 Race 2		Xv 82-8 Race 1	
	Lesions per 2 cm^2	Diameter per lesion (mm x 10)	Lesions per 2 cm^2	Diameter per lesion (mm x 10)	Lesions per 2 cm^2	Diameter per lesion (mm x 10)
F_2 population						
Susceptible[a]	18.3 ± 0.90[b]	3.9 ± 0.16	8.3 ± 1.06	4.3 ± 0.22	42.7 ± 1.63	7.6 ± 0.31
Remainder	4.2 ± 0.22	2.6 ± 0.05	2.7 ± 0.19	3.2 ± 0.08	37.7 ± 0.55	5.8 ± 0.10
t-test value	21.55**	7.04**	8.81**	5.18**	3.24**	6.31**
Backcross (F_1 x ECW)[c]						
Susceptible	26.7 ± 0.69	5.1 ± 0.25	17.3 ± 1.29	5.6 ± 0.24	41.7 ± 1.09	8.4 ± 0.51
Remainder	14.0 ± 0.40	4.2 ± 0.12	10.2 ± 0.54	5.0 ± 0.11	42.5 ± 0.61	7.6 ± 0.20
t-test value	15.41**	3.38**	5.97**	2.65**	0.68 ns[d]	1.61 ns

[a]Susceptible plants had numbers of lesions of isolate Xv 77-3A which were > the mean plus one standard deviation.
[b]Mean of the group ± standard error of mean.
[c]ECW = Early Calwonder.
[d]ns = Differences between means were not significant (P>0.05).
**Differences between means were significant at P<0.01.

Lesion diameter was greater in all generations with isolate Xv 77-3 than with isolate Xv 83-1. Inoculum concentration was slightly greater with isolate Xv 77-3 than Xv 83-1, and this was reflected in lesion numbers per unit area of leaf. The frequency distributions of lesion numbers and diameters were continuous in both populations, and no distinct segregation ratios were apparent. These observations do not differ from those with isolates Xv 77-3A, Xv 82-7, and Xv 82-8.

Lesion numbers with both isolates Xv 77-3 and Xv 83-1 were relatively large in plants of ECW and small in plants of 246-4 (Tables 7-12 and 7-13). With isolate Xv 83-1, the generation means of number of lesions paralleled very closely the pattern observed with the weak growing isolates Xv 77-3A and Xv 82-7 (Table 7-4), that is, lesion numbers of the F_1, F_2, and two backcrosses were between the two parental means but skewed toward the resistant parent, and the mean of the backcross (F_1 x ECW) closely approximated the average of the F_1 and ECW populations. In contrast to this, observed generation means of lesion numbers with isolate Xv 77-3 followed relatively closely those values expected for <u>additive</u> gene action, that is, F_1 and F_2 means approximated the mid-parent values, and the two backcross means approximated the averages of the F_1 and respective parental means.

Simple additive-dominance gene action models failed to fit adequately the weighted generation means of lesion numbers with both isolates Xv 77-3 and Xv 83-1 (Tables 7-12 and 7-13). With isolate Xv 77-3, however additivity and additive x dominance epistasis were the only significant genetic parameters, with additivity being the parameter of overriding importance. Similarly, additivity for lesion diameter with isolate Xv 83-1 was the major genetic parameter (Table 7-13).

Table 7-12. Analysis of weighted generation means of number of lesions per 2 cm^2 of leaf and diameter per lesion for progenies of peppers Early Calwonder and 246-4 inoculated with isolate Xv 77-3 of Xanthomonas campestris pv. vesicatoria.

	Lesions[a] per 2 cm^2 of leaf		Diameter per lesion (mm x 10)	
Generation	Mean	Variance of mean	Mean	Variance of mean
ECW[b]	40.9	1.54	6.1	0.027
246-4	4.4	0.13	1.6	0.004
F_1 (ECW x 246-4)	26.8	2.90	4.9	0.008
F_2	23.2	0.46	4.2	0.004
F_1 x ECW	35.3	0.53	5.6	0.005
F_1 x 246-4	14.1	0.45	2.9	0.006
Parameter[b]	Estimate	S.E. of estimate	Estimate	S.E. of estimate
m	16.7*	3.42	3.4*	0.34
a	18.3*	0.65	2.2*	0.09
d	16.0	8.43	1.5	0.85
aa	6.0	3.36	0.4	0.33
ad	6.0*	2.36	0.9*	0.27
dd	-5.9	6.02	-0.1	0.54
Goodness of fit test	Not applicable[e]		Not applicable	

[a]Inoculum concentration approximately 2.9 x 10^3 cfu ml^{-1}.
[b]ECW = Early Calwonder.
[c]Parameters: m = theoretical population mean at F∞; a = additivity; d = dominance deviation; aa = additive epistasis; ad = additive x dominance epistasis; dd = dominance epistasis.
[d]S.E. = standard error.
[e]All degrees of freedom used in parameter estimation.
*Significantly different from zero at $P<0.05$.

Table 7-13. Analysis of weighted generation means of number of lesions per 2 cm^2 of leaf and diameter per lesion for progenies of peppers Early Calwonder and 246-4 with isolate Xv 83-1 of Xanthomonas campestris pv. vesicatoria.

	Lesions[a] per 2 cm^2 of leaf		Diameter per lesion (mm x 10)	
Generation	Mean	Variance of mean	Mean	Variance of mean
ECW[b]	20.3	0.66	4.5	0.019
246-4	0.5	0.02	1.6	0.022
F_1 (ECW x 246-4)	1.6	0.07	3.1	0.055
F_2	2.5	0.05	2.4	0.005
F_1 x ECW	9.9	0.22	3.7	0.005
F_1 x 246-4	0.5	0.004	1.9	0.012
Parameter[c]	Estimate	S.E.[d] of estimate	Estimate	S.E. of estimate
m	-0.5	1.39	1.5*	0.39
a	9.9*	0.41	1.5*	0.10
d	9.5*	3.63	2.1	1.03
aa	10.8*	1.32	1.5*	0.38
ad	-1.1	1.26	0.7*	0.33
dd	-7.4*	2.32	-0.5	0.78
Goodness of fit test	Not applicable[e]		Not applicable	

[a]Inoculum concentration approximately 1.5 x 10^3 cfu ml^{-1}.
[b]ECW = Early Calwonder.
[c]Parameters: m = theoretical population mean at F∞; a = additivity; d = dominance deviation; aa = additive epistasis; ad = additive x dominance epistasis; dd = dominance epistasis.
[d]S.E. = standard error.
[e]All degrees of freedom used in parameter estimation.
*Significantly different from zero at $P<0.05$.

The interpretation of the analysis of generation means of lesion numbers with isolate Xv 83-1 (Table 7-13), like that with isolate Xv 77-3A (Table 7-7) was not adequate since the estimate of the general mean did not differ significantly from zero, or no disease.

The variation in generation means of lesion diameter for isolates Xv 77-3 and Xv 83-1 were very similar. In both cases, the F_1 and F_2 means approximated the mid-parent value, and the backcross means closely approximated the averages of the F_1 and respective parental means (Tables 7-12 and 7-13). However, simple additive-dominance gene action models again failed to fit the data, and full digenic models were necessary (Tables 7-12 and 7-13). Additive gene action, and not dominance or epistasis, was the genetic parameter of major importance for both isolates. These data were entirely consistent with the importance of additive gene action for control of lesion diameter with isolates Xv 77-3A, Xv 82-7, and Xv 82-8 (Tables 7-4, 7-7 and 7-8).

A pattern became clear from the analyses with five bacterial isolates to explain the inheritance of nonhypersensitive resistance in selection 246-4. Disease severity varied directly with the bacterial isolate. Resistance was greatest to the weak growing isolates, and least to the most aggressive isolate. Resistance did not appear to directly reflect differences among the three races of Xcv. Disease severity with each of the three races in the F_2 and backcross (F_1 x ECW) populations was correlated.

Additive gene action basically controlled lesion diameter with all bacterial isolates. The generation means of isolate Xv 82-8 exemplify the pattern of an aggressive isolate (Tables 7-4 and 7-8). Additive gene action implies a reduction in the rate of lesion expansion (that

is, bacterial multiplication) in linear proportion with frequency of the resistance allele(s). Bacterial multiplication in homozygous resistant plants is so restricted (Tables 7-1, 7-3, and 7-4) that very few necrotic areas become visible lesions. However, far more necrotic areas grow to become visible lesions when resistant plants are inoculated with an aggressive isolate (Table 7-4). The reduced rate of expansion of lesions in heterozygotes compared to homozygous susceptible plants is not sufficient to prevent all potential lesions of an aggressive bacterial isolate from becoming visible, but is sufficient to prevent most necrotic areas of weak isolates from becoming visible (Table 7-4). Consequently, reversal of dominance of apparent gene action controlling lesion numbers will occur in proportion with the aggressiveness of the bacterial isolates. Additive gene action may appear to control lesion numbers with bacterial isolates of intermediate aggressiveness. This also did occur (Table 7-12).

Additive epistasis appeared to be important in the genetic control of large lesion diameter with the less aggressive (Tables 7-7 and 7-13) but not the most aggressive bacterial isolates (Table 7-8). This also may be explained by resistance which controls a reduced rate of bacterial multiplication. Very few necrotic areas caused by weak bacteria enlarge to become visible. These few lesions are of small diameter. In plants with no alleles for resistance, many more necrotic areas enlarge to become visible lesions and their diameters are substantially greater (Table 7-4). This gives the appearance of epistasis for high values of lesion diameter. In contrast, almost all potential necrotic areas in heterozygotes and susceptible homozygotes inoculated with an aggressive isolate enlarge to become visible lesions (Table 7-4). Lesions in

susceptible homozygotes have larger diameter than those in heterozygotes and additive gene action with no epistasis is indicated (Table 7-8).

Strikingly similar results to these were obtained with nonhypersensitive resistance in other PI lines. Resistance in lines PI 163189 and 271322 could be attributed to single genes (Chapters 3 and 6 of this dissertation). There is no evidence in the present study to refute the notion that resistance in 246-4 is also controlled by a monogenic additive factor. Hibberd and Gillespie (1982a) also observed intermediate disease severity in field grown heterozygotes of a cross between susceptible bell pepper and PI 322719. Other reports of reversal of dominance and recessiveness in the inheritance of resistance to bacterial pathogens of plants are common in the literature. For example Brinkerhoff et al. (1984), Chand and Walker (1964a and b), Fallik et al. (1984), Innes et al. (1984), Kim (1983), Patel (1982), Patel and Walker (1966), Sidhu and Khush (1978), Taylor et al. (1978), and Yoshimura (1984) have reported them. It is possible that underlying additivity may be occurring with many quantitatively-assessed resistance genes (Valladares-Sanchez, 1979).

Efficient selection of genes for nonhypersensitive resistance in segregating populations is vitally important in plant breeding. Single plants with resistance were efficiently identified by inoculating fully expanded leaves with low concentration inoculum (Stall, 1981). The apparent dominance reversal of resistance is of concern, and several approaches to selection are possible. Commonly, inbred backcross progenies are inoculated (Brinkerhoff et al., 1984; Hibberd and Gillespie, 1982a; Innes et al., 1984; Patel, 1982; Stall, 1981), but two generations per cycle are required. Single generation cycles may be

accomplished by progeny testing presumed heterozygotes (Hanson, 1959). Alternatively, heterozygotes may be identified by inoculating with a relatively weak growing isolate.

Few data are available to indicate the degree of resistance necessary to be associated with a major yield advantage (Hibberd and Gillespie, 1982b). It is possible that hybrid cultivars heterozygous for nonhypersensitive resistance may be adequate for controlling bacterial spot in many environments. Although nonhypersensitive resistance in homozygous PI lines is highly stable in diverse environments, stability in heterozygotes has never before been examined.

CHAPTER 8
FIELD RESISTANCE TO BACTERIAL SPOT IN PEPPER CORRELATED WITH COMPONENTS OF RESISTANCE MEASURED IN GREENHOUSE PLANTINGS

Genes occur in pepper (Capsicum annuum L.) germplasm that control hypersensitive resistance to bacterial spot incited by Xanthomonas campestris pv. vesicatoria (Doidge, 1920), Dye 1978 (designated here as Xcv). These genes segregate independently, are dominant (Chapters 4 and 5, this dissertation), and are expressed at widely differing plant maturities (Chapter 3, this dissertation). They may be rapidly moved to bell pepper backgrounds by simple recurrent selection in small populations (Cook and Guevara, 1984; Cook and Stall, 1969; Kim and Hartmann, 1985). There is great potential for control of Xcv by these genes (Cook, 1977; Cook and Guevara, 1984).

Other germplasm of C. annuum has genes which control highly effective and stable resistance to bacterial spot. These genes control more typically nonhypersensitive resistances (Adamson and Sowell, 1983; Borchers, 1965; Greenleaf, 1960; Hibberd and Gillespie, 1982a; Kim, 1983; Sowell, 1960; Sowell and Dempsey, 1977; Stall, 1981). The degree of resistance in plants heterozygous for these genes varies with the culture of Xcv (Chapters 4, 6, and 7 of this dissertation) and the test environment (Hagedorn, 1982; Hibberd and Gillespie, 1982a). Early detection of these resistances can be achieved in greenhouse-grown tests of young plants in which leaves are infiltrated with a suitably low concentration of bacteria (Stall, 1981). Resistance was reflected in two components of resistance, namely, numbers of lesions per unit area of leaf, and lesion diameter. However, data were lacking to conclude that this resistance correlated well with field resistance.

This chapter reports a correlation between resistance to Xcv in field and greenhouse experiments.

Materials and Methods

Inoculum Preparation

The bacterial isolates represented all 3 races of Xcv (Chapter 4, this dissertation) and were stored in refrigerated sterilized water. Inocula were prepared from late log-phase, agitated cultures in nutrient broth. After centrifugation, bacterial pellets were resuspended in sterile tap water, and standardized colorimetrically to 50% light transmittance to approximate a density of 5×10^8 cfu (colony forming units) ml^{-1}. These suspensions were serially diluted to final concentrations, which ranged between 1.0×10^3 to 3.0×10^3 cfu ml^{-1}. Concentrations were confirmed by plating replicated 0.05 ml subsamples onto nutrient agar and counting colonies. The following bacterial isolates were used in these tests: Xv 71-21, Xv 80-5, and Xv 82-8 of race 1, Xv 82-7 and Xv E3 of race 2, and Xv 69-1 and Xv 77-3A of race 3. The race of each bacterial culture was verified by reaction to inoculation with 5×10^8 cfu ml^{-1} of pepper plants with genes Bs_1 and Bs_3. These genes control race specific hypersensitivity (Chapter 4, this dissertation). All inoculations were by hypodermic infiltration of intercostal leaf tissues.

Seed Sources

Seed of plant introductions (PI) of *C. annuum* and *C. chinense* and breeding lines of *C. annuum* were obtained from several sources. The PI lines were from the USDA South Atlantic Regional Plant Introduction Station, Experiment, Georgia, and their numbers were 163184, 163189, 163192, 164471, 164677, 173877, 182646, 183439, 183440, 183441, 183922, 215740, 244670, 246331, 271322 of *C. annuum*, and 260509, 281423,

281444 of C. chinense. Four related inbred breeding lines (5-1-1-10, 5-1-1-LF, 38-13, 44-1-3), which were selected in greenhouse plantings of segregating progenies of the cross between PI 271322 and the bell cultivar Early Calwonder (ECW) (Stall, 1981), were included together with two unrelated inbred breeding lines, namely 171-3 from the cross between PI 322719 and Yolo Wonder (Hibberd and Gillespie, 1982a), and C7-1 from the cross between cultivars Hungarian Yellow and Yolo Wonder (Hibberd and Gillespie, 1982b). All breeding lines were inbred to the equivalent of F_4 or F_5. Control cultivars were Florida VR-4 with the Bs_1 gene for hypersensitive resistance to race 2 of Xcv (A.A. Cook, 1982, personal communication) and ECW with no genes for hypersensitive resistances.

Greenhouse Experiments

Several experiments were completed in a greenhouse to compare the degree of resistance in most lines with controls. The PI lines were compared with ECW for resistance to all 3 races. Population sizes varied (Table 8-1). Resistant plants were selected from each line and inbred once. Several selections were made from 2 lines, namely PI 163189 and 271322. Progeny of each selection and breeding lines C7-1 and 171-3 were compared with ECW for resistance to an isolate, Xv 77-3A, of race 3 in an experiment comprising 2 replicates of 5 plants each. Race 3 was chosen for this test because genes for typical hypersensitive resistance to this race did not occur in any plant (Cook and Stall, 1969; Kim, 1983; Chapters 3 and 6 of this dissertation). The Florida breeding line 44-1-3 (Stall, 1981) was tested against ECW for resistance to race 1 (isolate Xv 82-8) and race 2 (isolate Xv 82-7) in specific comparisons. Mean values in the several experiments were compared by t-test or Duncan's multiple range test. All plants were raised in

steamed peat-vermiculite mix in 10-cm plastic pots arranged in randomized rows of 8 plants. They were watered as required and treated four times during the experiments with approximately 0.4 g per pot of soluble 20:20:20 fertilizer.

Assessment of Disease Severity in Greenhouse Experiments

Segments of fully expanded leaves adjacent the first fork on the main stem were inoculated with low concentrations of each of the isolates Xv 82-8 (race 1), Xv 82-7 (race 2), and/or Xv 77-3A (race 3). Leaves were collected approximately 3 weeks after inoculation. Time of sampling varied with the experiment and depended on the rate of lesion area expansion in susceptible control lines. In all experiments, sampling was timed to occur while lesions remained discreet. The number of lesions within 2 cm^2 of leaf imprinted by a cork-borer was counted at each inoculation site viewed under a dissecting microscope (magnification 2.5 X). The diameters of five lesions at random at each site, or of all lesions where fewer than five existed were measured using a graduated eyepiece. Mean values were computed on a plant basis.

Bacterial Populations in vivo

Low disease severity in PI lines was expected to correlate with low bacterial populations in vivo. Seedlings of inbred progeny of single plant selections from PI 163189, 173877, 244670, 246331, 271322, and control line ECW were raised in a greenhouse as described above. Three fully expanded leaves per plant were each inoculated with each of the isolates Xv 80-5 (race 1), Xv E3 (race 2), and Xv 69-1 (race 3). Leaves from single plants were harvested at 2, 5, 8, 11, and 14 days after inoculation. Bacterial populations were determined from replicated 1.0 cm^2 (i.e., 2 x 0.5 cm^2) leaf samples. Samples were

triturated in 0.5 ml sterile water, the suspensions diluted where appropriate, and 0.05 ml subsamples of the final dilutions spread on nutrient agar plates. Colonies were counted after 2 to 3 days incubation at 30 C and mean values converted to $\log_{10}$ (cfu cm^2) of leaf. The experiment was repeated.

Field Resistance

Field resistance of selections from PI lines, breeding lines, and control cultivars was evaluated in a replicated planting at Gainesville, Florida. Container grown seedlings were raised. Prior to transplanting, a single leaf per seedling was inoculated with a mixture containing approximately 2.5×10^3 cfu ml^{-1} of each of 4 isolates. Isolates used were Xv 71-21 and Xv 82-8 of race 1, Xv E3 of race 2, and Xv 77-3A of race 3. Seedlings were transplanted in mid-June 1983 to raised beds on 1.3 m centers. Intra-row spacing was 0.3 m. There were 3 replicates of single row plots of 12 plants. The percentage of diseased leaf tissue was rated seven times at weekly intervals. Leaves fallen in response to bacterial spot were included in each assessment. Ratings were made four times on a single plant basis, and plot means were computed. Three ratings were made on an entire plot basis when interplant contact was extensive. Ratings were ceased when Cercospora leaf spot became incident and resulted in leaf fall. The cumulative proportion of diseased leaf was converted to area under the disease progress curve, and entry means were compared by Duncan's multiple range test.

Results

Resistance to Xcv in Greenhouse Plantings

All PI lines tested were resistant to three races of Xcv in comparison with the control cultivar, ECW in an initial greenhouse planting

(Table 8-1). The most resistant PI lines were 271322, 244670, and 163189 of C. annuum, and 281423 and 281444 (C. chinense). Few lesions of small diameter occurred per 2 cm^2 of leaf in these lines. Lesions were of uniform, very small diameter in PI 271322 but of variable size in other lines. Few lesions occurred in PI 260509 (C. chinense), but lesions were relatively large. In other lines greater variability occurred in lesion numbers than in lesion diameters. Plants with low values of both components were selected from all lines, and several selections were made from PI's 163189 and 271322.

Significant variation in components of number of lesions per 2 cm^2 of leaf and lesion diameter occurred among entries of ECW, 171-3, C7-1, and inbred PI lines inoculated with isolate Xv 77-3A of race 3 (Table 8-2). Relatively many lesions of large diameter occurred in ECW. Most other lines and 171-3 had very small lesions but greater variability was found in lesion numbers. The phenotypic correlation between the two components of resistance was $r_p = 0.76$. Thus, 58% of the phenotypic variation in lesion number in these data was associated with variation in lesion diameter. Mean values of the two components were nonlinearly related, however (Figure 8-1) such that a large increase in number of lesions per 2 cm^2 of leaf was associated with a very small increase in in lesion diameter among inbred PI lines. A further but relatively small increase in lesion numbers in C7-1 and ECW was associated with a large increase in lesion diameters in those lines compared with inbred PI lines. The line C7-1 had values of both components of resistance which were greater than in PI lines and 171-3 but lower than in ECW.

The Florida breeding line 44-1-3 was compared with ECW for resistance to races 1 and 2 in a replicated test. Few lesions of small

Table 8-1. Lesions per 2 cm^2 of leaf and diameter per lesion at 32 days after inoculating pepper plant introductions and control cultivar with races 1, 2, and 3 of Xanthomonas campestris pv. vesicatoria.

Host line	Number of plants	Bacterial isolate and race designation					
		Xv 82-8; race 1		Xv 82-7; race 2		Xv 77-3A; race 3	
		Lesions per 2 cm^2	Diameter per lesion (mm)	Lesions per 2 cm^2	Diameter per lesion (mm)	Lesions per 2 cm^2	Diameter per lesion (mm)
PI 163184	17	21.2 ± 3.07[b]	0.23 ± 0.03	17.6 ± 2.45	0.28 ± 0.03	17.9 ± 4.55	0.28 ± 0.05
PI 163189	18	18.0 ± 4.70	0.17 ± 0.01	4.7 ± 1.56	0.14 ± 0.01	10.1 ± 2.83	0.14 ± 0.01
PI 163192	16	38.3 ± 4.83	0.29 ± 0.03	11.8 ± 1.65	0.25 ± 0.03	17.8 ± 3.54	0.19 ± 0.02
PI 164471	19	30.1 ± 3.02	0.33 ± 0.03	11.4 ± 2.07	0.22 ± 0.03	13.0 ± 0.22	0.22 ± 0.03
PI 183440	6	32.5 ± 3.17	0.24 ± 0.07	13.0 ± 5.35	0.18 ± 0.17	15.5 ± 6.90	0.21 ± 0.08
ECW[c]	5	32.5 ± 9.32	0.88 ± 0.06	22.7 ± 4.63	1.03 ± 0.04	22.3 ± 6.10	0.88 ± 0.37
PI 164677	10	8.2 ± 1.03	0.15 ± 0.02	5.7 ± 1.91	0.16 ± 0.02	9.0 ± 3.92	0.14 ± 0.01
PI 173877	15	10.9 ± 2.00	0.26 ± 0.04	5.6 ± 1.76	0.27 ± 0.05	10.8 ± 2.91	0.21 ± 0.03
PI 182646	17	13.5 ± 1.67	0.34 ± 0.03	9.2 ± 1.65	0.33 ± 0.03	19.7 ± 3.06	0.30 ± 0.04
PI 183439	17	14.8 ± 1.00	0.26 ± 0.03	9.2 ± 1.80	0.29 ± 0.04	15.6 ± 4.23	0.27 ± 0.04
PI 183441	12	7.2 ± 3.32	0.21 ± 0.03	3.8 ± 0.97	0.10 ± 0.02	5.5 ± 1.54	0.18 ± 0.02
ECW	4	23.7 ± 4.08	0.70 ± 0.04	18.0 ± 7.18	0.80 ± 0.09	52.8 ± 11.57	0.70 ± 0.03
PI 183922	19	15.6 ± 2.55	0.28 ± 0.03	9.6 ± 2.13	0.21 ± 0.02	13.2 ± 2.72	0.20 ± 0.02
PI 244670	20	10.1 ± 2.51	0.17 ± 0.02	4.8 ± 1.47	0.19 ± 0.02	4.5 ± 1.45	0.13 ± 0
PI 246331	6	12.0 ± 1.54	0.19 ± 0.04	11.6 ± 5.56	0.24 ± 0.08	14.2 ± 6.78	0.13 ± 0.05
PI 271322	15	1.1 ± 0.40	0.10 ± 0	1.8 ± 0.46	0.10 ± 0	4.9 ± 0.99	0.10 ± 0
ECW	5	25.3 ± 5.50	0.84 ± 0.04	20.7 ± 5.15	0.93 ± 0.04	28.5 ± 7.25	0.79 ± 0.05

PI 215740	20	14.1 ± 1.31	0.24 ± 0.01	6.5 ± 0.86	0.25 ± 0.02	11.9 ± 2.08	0.29 ± 0.03
PI 260509	17	6.4 ± 1.67	0.32 ± 0.06	6.7 ± 1.70	0.42 ± 0.08	7.5 ± 2.25	0.40 ± 0.05
PI 281423	10	6.5 ± 1.73	0.18 ± 0.02	1.9 ± 0.87	0.20 ± 0.06	1.9 ± 0.73	0.15 ± 0.03
PI 281444	13	2.6 ± 0.76	0.15 ± 0.61	3.5 ± 1.74	0.23 ± 0.05	2.5 ± 1.24	0.27 ± 0.11
ECW	4	20.8 ± 2.51	0.80 ± 0.10	26.5 ± 5.92	1.20 ± 0.26	23.5 ± 2.13	0.77 ± 0.08

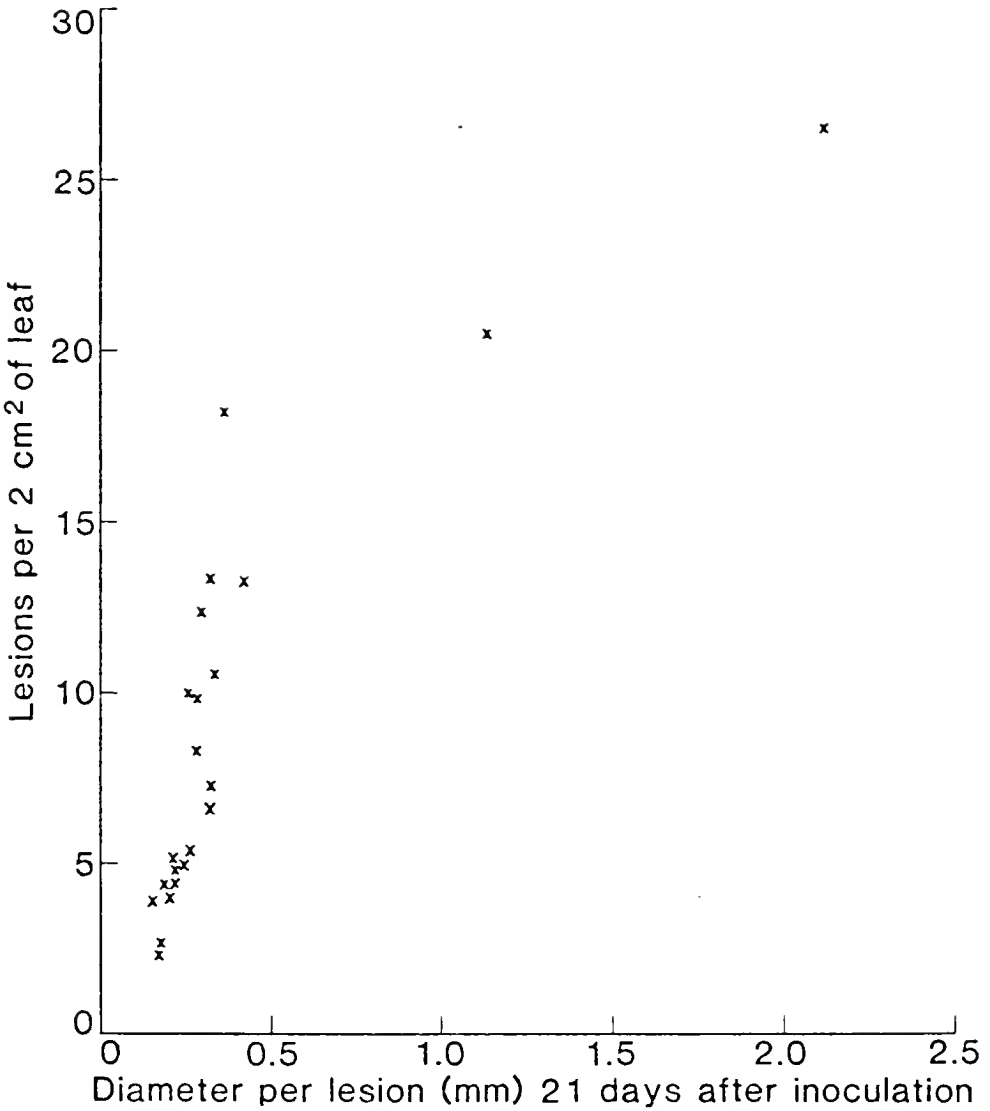
[a]Inoculum concentration approximately 2.5×10^3 cfu ml^{-1}.
[b]Mean ± standard error of mean.
[c]ECW = Early Calwonder.

Table 8-2. Lesions per 2 cm^2 of leaf and diameter per lesion 21 days after inoculating inbred pepper plant introductions, breeding lines, and control cultivar with race 3 of Xanthomonas campestris pv. vesicatoria.

Host line	Lesions per 2 cm^2 of leaf[a]	Diameter per lesion (mm x 10)
ECW[b]	26.5 u[d]	2.11 x
C7-1[c]	20.5 u v	1.14 y
164471-21	18.2 v w	0.35 y
215740-2	13.3 v w x	0.43 y
182646-11	13.3 v w x	0.31 y
163184-1	12.4 w x y	0.29 y
171-3	10.6 x y z	0.33 y
271322-1	10.0 x y z	0.26 y
164677-21	9.7 x y z	0.28 y
183439-19	8.4 x y z	0.28 y
271322-12	7.3 x y z	0.32 y
173877-9	6.6 x y z	0.31 y
260509-14	5.4 y z	0.27 y
163192-16	5.1 y z	0.22 y
163189-19	5.0 y z	0.23 y
163189-5	4.9 y z	0.22 y
183440	4.5 z	0.22 y
183441-6	4.4 z	0.19 y
271322-13	4.1 z	0.20 y
183922-11	3.9 z	0.15 y
246331-4	2.6 z	0.18 y
244670-7	2.4 z	0.17 y

[a]Inoculum concentration of isolate Xv 77-3A was 1.9×10^3 cfu ml^{-1}.
[b]ECW = Early Calwonder.
[c]Breeding line.
[d]Means not followed by same letter (i.e. u through z) differ significantly at $P<0.05$.

Figure 8-1. Relation between number of lesions per 2 cm^2 of leaf and diameter per lesion in resistant and susceptible peppers inoculated with race 3 of Xanthomonas campestris pv. vesicatoria.


30
25
20
15
10
5
0
Lesions per 2 cm2 of leaf
0
0.5
1.0
1.5
2.0
2.5
Diameter per lesion (mm) 21 days after inoculation

diameter occurred with both isolates in leaves of 44-1-3 compared with many, relatively large lesions in ECW (Table 8-3). Lesion diameter increased rapidly over time in ECW, and those lesions induced by isolate Xv 82-7 of race 2 unexpectedly became confluent in ECW and could not be counted 12 days after inoculation.

Bacterial Populations in vivo

Populations of bacteria in vivo were estimated in leaves of inbred selections of a sample of PI lines and control cultivar ECW inoculated with low concentrations of isolates of races 1, 2, and 3. Bacteria of all races multiplied in ECW to 10^7 to 10^8 cfu cm^{-2} of leaf in 12 to 14 days (Table 8-4). In contrast, bacteria multiplied in leaves of resistant PI lines after inoculation, and reached maximum numbers of approximately 10^3 to 10^4 cfu cm^{-2} of leaf at day 5; and numbers slowly declined thereafter. Lowest populations occurred in selections of PI 271322 and 246331. Lesions of isolates of all 3 races in plants of ECW became visible between 5 and 6 days after inoculation corresponding to populations of approximately 10^6 cfu cm^{-2} of leaf. Occasional isolated lesions of very small size were observed in plants of PI lines.

Field Resistance to Xcv

Wide variation occurred among entries for disease severity assessed as area under the cumulative disease progress curve (Table 8-5). Cultivars Florida VR-4 and ECW were susceptible. Intermediate disease severity occurred on breeding lines C7-1 and EC 38-13. The breeding lines 171-3 and EC 44-1-3 were resistant, but had nonsignificantly greater disease severity than the majority of inbred PI lines and breeding lines 5-1-1-10 and 5-1-1-LF. The lines 246331-4, 183922-11, 271322-1, 5-1-1-LF, 244670-7, 183441-6, 173877-9, 271322-13,

Table 8-3. Lesions per 2 cm^2 of leaf and diameter per lesion in two pepper lines at timed intervals after inoculation with races 1 and 2 of Xanthomonas campestris pv. vesicatoria.

Bacterial culture	Host	Days after inoculation[a] 6	8	10	12	14	17	20
Race 1 isolate Xv 82-8	ECW[b]	14.8 ± 5.6[c]	6.8 ± 0.9	19.7 ± 0.8	24.3 ± 4.4	26.8 ± 1.9	22.2 ± 2.7	29.3 ± 3.6
		0.5 ± 0.16	0.3 ± 0.01	0.5 ± 0.04	0.8 ± 0.07	0.8 ± 0.18	0.7 ± 0.07	1.0 ± 0.07
	44-1-3	4.5 ± 0.24	2.0 ± 0.3	4.0 ± 1.4	3.5 ± 0.9	2.5 ± 0.5	5.2 ± 1.0	7.7 ± 3.5
		0.2 ± 0.13	0.2 ± 0.03	0.3 ± 0	0.3 ± 0	0.3 ± 0	0.3 ± 0.03	0.3 ± 0.03
Race 2 isolate Xv 82-7	ECW	15.8 ± 6.2	6.8 ± 3.0	20.0 ± 3.5	22%[d]	30%[d]	40%[d]	-
		0.3 ± 0.02	0.3 ± 0.03	0.3 ± 0.04	-	-	-	-
	44-1-3	1.0 ± 0.07	0.3 ± 0.3	0.8 ± 0.6	5.3 ± 2.2	10.0 ± 4.0	11.3 ± 2.9	-
		0.3 ± 0	0.3 ± 0	0.3 ± 0	0.3 ± 0	0.3 ± 0	0.3 ± 0	-

[a]Inoculum concentrations were 2.1 x 10^3 cfu ml^{-1} and 2.3 x 10^3 cfu ml^{-1} for races 1, and 2, respectively.
[b]ECW = Early Calwonder.
[c]Upper value is the number of lesions per 2 cm^2 of leaf, and lower value is diameter per lesion (mm) ± standard error of mean.
[d] Lesions were confluent and impossible to count. Values are means of estimated percent necrosis.

Table 8-4. Estimated bacterial populations in leaf samples from six pepper lines at timed intervals after inoculating with races 1, 2, and 3 of Xanthomonas campestris pv. vesicatoria.

Bacterial culture	Host line	Log_{10} of bacteria per cm^2 of leaf Days after inoculation 2	5	8	11	14
Race 1[a]	ECW[b]	2.65[c]	6.16	6.83	7.52	7.99
isolate Xv 80-5	163189-5	2.60	4.65	4.03	4.02	4.87
	173877-9	2.74	4.17	2.79	3.22	2.57
	244670-7	2.57	4.49	4.43	3.82	2.93
	246331-4	2.69	3.04	3.14	3.52	1.77
	271322-4	2.40	2.24	1.30	0.70	1.00
Race 2	ECW	3.69	6.49	7.35	7.30	7.76
isolate Xv E3	163189-5	3.23	3.14	2.41	3.29	3.34
	173877-9	3.21	4.43	2.53	3.84	3.66
	244670-7	2.48	4.49	5.19	1.23	1.51
	246331-4	2.00	4.53	1.84	3.54	1.06
	271322-4	2.51	4.17	1.80	2.65	1.81
Race 3						
isolate Xv 69-1	ECW	2.73	5.69	6.70	6.93	7.33
	163189-5	2.30	3.91	3.56	2.93	1.70
	173877-9	2.36	4.23	3.75	1.08	2.73
	244670-7	2.33	4.23	1.86	0.54	1.13
	246331-4	1.90	3.36	2.20	0.48	1.64
	271322-4	2.75	3.37	3.31	3.10	0.88

[a]Inoculum concentrations of 1.4×10^3, 2.2×10^3, and 0.8×10^3 cfu ml^{-1} of races 1, 2, and 3, respectively.

[b]ECW = Early Calwonder.

[c]Values are means of 6 replicates, excepting 3 replicates for 163189-5.

Table 8-5. Disease severity (area under disease progress curve) in field planted pepper lines inoculated with three races of Xanthomonas campestris pv. vesicatoria.

Host line	Area under disease progress curve[a]	
Fla. VR-4	9.860	v[d]
ECW[b]	8.234	w
C7-1[c]	4.224	x
EC 38-13[c]	2.452	y
281423-2	1.036	z
171-3[c]	0.452	z
EC 44-1-3[c]	0.423	z
183440	0.246	z
281444-10	0.231	z
182646-11	0.227	z
260509-14	0.200	z
167184-1	0.191	z
163192-16	0.181	z
271322-12	0.181	z
163189-19	0.179	z
183439-19	0.178	z
164471-21	0.112	z
5-1-1-10[c]	0.083	z
271322-13	0.071	z
173877-9	0.069	z
183441-6	0.068	z
244670-7	0.053	z
5-1-1-LF[c]	0.052	z
271322-1	0.049	z
183922-11	0.045	z
246331-4	0.005	z

[a]Disease severity assessed as proportion of leaf tissue infected, including senesced leaves.
[b]ECW = Early Calwonder.
[c]Breeding lines.
[d]Means followed by different letters (v to z) differ significantly at $P<0.05$.

and 5-1-1-10 were all very highly resistant, and were essentially disease free. The C. chinense lines 260509-14, 281423-2 and 281444-10 appeared slightly but nonsignificantly more diseased than the majority of resistant C. annuum entries. Rapid yellowing and senescence of the few infected leaves occurred in breeding line 171-3, and this probably contributed to disease severity slightly greater than in most inbred PI lines. Similar rapid senescence did not occur in other resistant lines.

The simple correlations between area under the disease progress curve (Table 8-5), and number of lesions per unit area of leaf and diameter per lesion (Table 8-2), are $r = 0.79$ ($P<0.05$) and $r = 0.99$ ($P<0.01$), respectively. For the greenhouse experiment, the number of lesions per 2 cm^2 of leaf and diameter per lesion (Table 8-2) were converted to area of diseased tissue on the generally valid observation of circular lesions. The simple correlation between this diseased area and cumulative area under disease progress curve from field planting the same lines was $r = 0.96$.

Discussion

The PI lines resistant to all 3 races of Xcv were those described by Sowell (1960) and Sowell and Dempsey (1977). Many have now proven resistant in diverse environments (Borchers, 1965; Greenleaf, 1960; Hibberd et al., 1979; Kim, 1983; Stall, 1981). In the present tests, two of the most highly resistant lines, PI 163189 and 271322, were heterogeneous for 2 and 3 different resistance genes, respectively (Chapters 3 and 6 of this dissertation). These genes appear to have contributed to high ranking of resistance. Nevertheless, progeny of single plant selections from the other non-hypersensitively resistant PI lines subsequently proved to be the equal of PI 163189 and 271322 in greenhouse and field experiments.

Lesion diameter and lesion number per unit area of leaf in greenhouse plantings accurately reflected populations of bacteria in leaf mesophyll. Lesion diameter was the attribute more strongly associated with field resistance. This component probably reflects the extent of bacterial multiplication at local infection sites more completely than number of lesions per unit area (Figure 8-1) because the potential number of lesions per unit area is established by the inoculum density (Essenberg et al., 1979; Stall, 1981; Stall et al., 1982). Bacteria introduced into mesophyll multiply in situ to populations that are controlled, in part, by the resistance mechanism functioning in the host (Chapter 4; this dissertation). A small increase in bacterial populations may be associated with a large increase in the number of lesions which become visible (Figure 8-1). Lesion expansion will occur whether few or all of the potential lesions have become visible. The rate of lesion expansion will vary with the degree of resistance. Resistance should be evaluated by both the components lesion diameter and lesion numbers.

The breeding lines 171-3 and C7-1 were selected in Queensland for strong resistance (Hibberd and Gillespie, 1982a) and partial resistance respectively (Hibberd and Gillespie, 1982b) in comparison with susceptible control cultivars. These, and other breeding lines selected in greenhouse plantings in Florida (Stall, 1981) for resistance to race 1 maintained their relative rankings in these tests with all three races. This implies that resistance is stable, durable, and of high heritability. Prospects for longterm control of bacterial spot are therefore encouraging.

CHAPTER 9
DISCUSSION

The plant introduction (PI) lines of pepper (Capsicum annuum and C. chinense) used in these experiments were described by Sowell as resistant to bacterial leaf spot, incited by Xanthomonas campestris pv. vesicatoria (Doidge, 1920) Dye, 1978 (Xcv) (Sowell, 1960; Sowell and Langford, 1963; Sowell and Dempsey, 1977). These reports have now been confirmed by plantings in diverse environments (Borchers, 1965; Hibberd and Gillespie, 1982a; Hibberd et al, 1979; Kim, 1983; Kim and Hartmann, 1985). More recently, additional accessions of C. annuum were evaluated for resistance to Xcv (Kim, 1983), and resistance was detected and characterized as well in C. chaconese (Cook and Guevara, 1982; 1984). It is hoped that enthusiasts of Capsicum will continue to devote time to evaluation of germplasm for the discovery of some unique genes for resistance to bacterial spot. The genus Capsicum is diverse but several species are sufficiently compatible to allow gene transfer at the diploid level by conventional breeding (Cook and Guevara, 1984; Pickersgill, 1971).

Two types of genetic resistance to Xcv were evaluated in these experiments. These are the typical hypersensitive reaction (HR), and a form of reaction that superficially is not typically hypersensitive. The latter resistance is referred to here as nonhypersensitive. The difference between the reactions was detected first by observing leaves infiltrated with high concentrations of bacteria (Cook and Stall, 1969; Kim and Hartmann, 1985; Chapters 3 and 4 of this dissertation). Confluent necrosis developed within 24 h after inoculation with HR but necrosis required 2 to 3 days for nonhypersensitive resistance. Both reactions

differed from the necrosis occurring in the susceptible reaction (Chapters 3, 6, and 8 of this dissertation).

Comparison of Hypersensitive and Nonhypersensitive Resistances

The differences between typical hypersensitive and nonhypersensitive resistances are not clear. Differentiation of the two has been a source of confusion throughout these and other studies (Adamson and Sowell, 1983; Cook and Stall, 1969; Sowell and Dempsey, 1977; G. Sowell, Jr., 1982, personal communication). Nonhypersensitive resistance was examined in several PI lines in the present experiments. The reaction was compared with typical HR in terms of loss of electrolytes from inoculated leaf tissues, bacterial population *in vivo*, disease symptom development, and inheritance of resistance.

Several attributes were similar to HR. Bacterial multiplication ceased abruptly in leaves by 3 to 4 days after inoculation with low populations of bacteria (Chapters 3, 4, 6, 7, and 8 of this dissertation). Bacterial populations then declined and were no different from those with typical HR by 10 to 14 days after inoculation. Concomitantly, disease symptoms ceased developing in the manner expected of HR, that is, only a few small lesions appeared several days after inoculation and disease severity did not change subsequently (Essenberg et al., 1979; Klement, 1982; Klement et al., 1964; Turner and Novacky, 1974). In contrast, lesion numbers and sizes in susceptible plants continued increasing for about three weeks after inoculation.

Several features of nonhypersensitive resistance in PI 271322 served to distinguish it from typical HR. Plasmolysis of leaf tissue inoculated with high concentrations of bacteria was reversible in the

nonhypersensitive reaction. Most inoculated tissue (>90%) recovered after several hours of severe wilting and appeared relatively healthy (Chapter 4, this dissertation). Concomitantly, loss of electrolytes from inoculated tissues increased slightly. This contrasted with typical HR in which leaf tissues did not recover turgidity but collapsed, became necrotic and lost large quantities of electrolytes. Jones and Fett (1985) observed similar reversible plasmolysis of soybean leaves inoculated with one isolate of the bacterial blight pathogen, and regarded it as a form of HR. Ultrastructural studies of the nonhypersensitive reaction to Xcv in C. annuum may help to resolve the present confusion surrounding this form of resistance.

Variability in the time to both host cell collapse and maximum loss of ions from inoculated leaf tissues does exist among the four confirmed HR reactions to Xcv in pepper. The HR controlled by Bs_1 with race 2 is the most rapid, and HR to the tomato strain develops the slowest. Times to reaction controlled by Bs_2 with races 1 and 3, and Bs_3 with race 1 are intermediate (Cook, 1973; Cook and Guevara, 1984; Chapters 4 and 5, this dissertation). The nonhypersensitive resistance described in these chapters may be a fifth form of HR which is the slowest of all in developing. Similar variation in time to host cell collapse occurs among race specific genes controlling HR to fungi (Day, 1974).

A second difference between typical HR and nonhypersensitive resistance in PI 271322 was the rate at which bacteria died in leaf mesophyll after the resistances had been induced. Resistance controlled by genes for HR became effective by two days, and by nonhypersensitive resistance by five days after inoculation of plants with low

concentrations of bacteria (Chapter 4, this dissertation). Slow decline of bacterial populations then occurred with typical HR and rapid decline with nonhypersensitive resistance. Phytoalexins may be more involved in the nonhypersensitive resistance than in HR. Phytoalexins can accumulate to high levels and play a role in resistance (Keen, 1982). Greater accumulation may occur in resistance reactions which require a longer time to be induced (Ersek and Hevesi, 1983; Long et al., 1985). Once released, phytoalexins may play a greater role in debilitating bacteria in this reaction than with typical HR. No effort was made to quantify the relative roles of phytoalexin in resistance reactions described herein, although it would seem to be a profitable exercise.

A third difference between typical HR and nonhypersensitive resistance reactions involved their inheritance. Three genes for HR ($\underline{Bs_1}$, $\underline{Bs_2}$, and $\underline{Bs_3}$) segregated independently in crosses (Chapters 3 and 5 of this dissertation). Both the genes $\underline{Bs_1}$ and $\underline{Bs_3}$ were incompletely dominant in heterozygotes. Penetrance with $\underline{Bs_3}$ was greater than with $\underline{Bs_1}$ in terms of two parameters, namely bacterial populations *in vivo* and time to maximum loss of electrolytes from inoculated leaves (Chapter 4, this dissertation). The gene $\underline{Bs_2}$ in heterozygotes was not tested by similar parameters. Nevertheless, disease severity in $\underline{Bs_2}$ heterozygotes was very low (Chapter 5, this dissertation). The HR phenotypes induced by all three genes are clearly qualitative, dominant traits that are amenable to easy selection in backcross breeding progenies.

In contrast to this, nonhypersensitive resistance is quantitative, and inherited as a monogenic trait under control of additive gene action (Chapters 3, 5, 6, and 7 of this dissertation), that is, disease

severity in heterozygotes was intermediate to homozygotes. Evidence of monogenic, additive inheritance was found in progenies of two PI lines 163189 and 271322 crossed with susceptible bell pepper. The available evidence indicates similar inheritance in PI 246331.

Inheritance of Nonhypersensitive Resistance

Evidence for monogenic inheritance of nonhypersensitive resistance was most clear in segregating progenies derived from crossing the susceptible cultivar Florida VR-4 with PI 163189 (Chapter 6, this dissertation). Similar evidence was more difficult to obtain in progenies derived from crossing PI 271322 and 246331 with cultivar Early Calwonder (ECW) (Chapters 3, 5 and 7 of this dissertation).

The choice of the susceptible parent likely influenced detection of segregation for resistance. Cultivar ECW is recognized by farmers as often being slightly less severely diseased with bacterial spot than cultivars of the Yolo Wonder background, exemplified by Florida VR-4 (S. Subramanya, 1982, personal communication). Florida VR-4 was diseased significantly more severely than ECW in the field trial (Chapter 8, this dissertation). In addition, bacterial spot lesions expanded to become confluent more quickly in plants of Florida VR-4 (and susceptible progenies derived from it) than in plants of ECW and its susceptible progenies (compare Chapters 3, 5, and 7 with Chapter 6 of this dissertation). The difference in disease severity between resistant and susceptible plants in progenies of ECW may have been smaller than in Florida VR-4 progenies so that decisive monogenic segregation was more difficult to recognize. Florida VR-4 or a similar cultivar may have been the more appropriate choice for a susceptible parent in all these experiments.

Additional evidence to support this assumption occurred in progenies of the cross between ECW and PI 246331 (Chapter 7, this dissertation). Slight overdominance and possible transgressive F_2 segregation may have occurred for high lesion numbers with the aggressive isolate Xv 82-8 in this experiment. However, seed from severely diseased F_2 plants was not retained for further testing. Curiously, similar overdominance for numbers of lesions was noted in the data of Stall (1981) in progenies of PI 271322 also crossed with ECW. A slight degree of resistance may have been transferred to progeny from ECW plants.

Evidence of monogenic inheritance of resistance in progenies from the cross between PI 271322 and ECW occurred most clearly in second backcross and inbred backcross progenies in the present experiments (Chapter 3, this dissertation). The inbred-backcross method of determining the number of genetic factors controlling a trait (Wehrhahn and Allard, 1965) would appear to be well suited to studies of quantitative-assessed disease resistance.

It should be noted that intermediate disease severity in heterozygotes occurred also in other studies in pepper (Hibberd and Gillespie, 1982a), cucumber (Chand and Walker, 1964a and b; Dessert et al., 1982), bean (Innes et al., 1984; Patel and Walker, 1966; Taylor et al., 1978; Valladares-Sanchez, 1979), tomato (Fallik et al., 1984), and rice (Sidhu and Khush, 1978). It is likely that these examples also reflect additive gene action in the control of resistance to phytopathogenic bacteria.

Race Differentiation in Xcv

Nonhypersensitive resistance was effective against isolates of all three races of Xcv virulent on pepper (Chapters 3, 5, 6, 7, and 8 of this dissertation). One gene (designated Bs_4 in PI 271322) or a compound locus may occur in each PI line to control resistance. Races of Xcv could not be differentiated by this form of resistance. Tests for allelism of these genes in PI lines were not made, but genes may not be allelic (Adamson and Sowell, 1983). This resistance controlled a reduced rate of lesion expansion in inoculated tissues, that is, bacterial multiplication was greatly reduced. This occurred with all bacterial isolates tested, but the magnitude of the resistance varied with the isolate. Isolates could be grouped according to their vigor of lesion growth, or aggressiveness, in susceptible plants. Differences among isolates for lesion diameter in a given time were consistent across all generations of crosses between resistant and susceptible peppers grown in greenhouse environments. Epidemiologically, this resistance controlled a rate of field disease progress which was essentially zero in most resistant PI lines (Chapter 8, this dissertation).

The gene Bs_2, transferred by Cook from *C. chacoense* to *C. annuum*, controls hypersensitive resistance to races 1 and 3 (Chapter 5, this dissertation). Data to prove the observation (Cook, 1977) that Bs_2 also controls HR to race 2 is currently not available. However it is possible that Bs_2 is effective against all three races of Xcv and that races also may not be differentiated. The locus Bs_2 may be a rare example of interspecific gene transfer that has rendered a pathogenic bacterium nonpathogenic (Klement, 1982).

From preliminary observation, $\underline{Bs_2}$ may be a compound locus within which genetic recombination can occur. Recombinants may be recovered in the future to differentiate among races of Xcv. A possible recombinant plant with greater disease severity than expected was observed (Chapter 5 of this dissertation), but this plant reaction may have resulted from localized environmental conditions. Natural watersoaking of leaf mesophyll may have occurred unequally among plants in the greenhouse. Watersoaking inhibits HR that is expected to occur in plants with $\underline{Bs_1}$, $\underline{Bs_2}$, or $\underline{Bs_3}$ inoculated with bacteria of the appropriate races (Stall and Cook, 1979; and personal observations). This may have resulted in unusually severe disease in one $\underline{Bs_2}$ heterozygote (Chapter 5, this dissertation).

There are similarities between HR to the pepper strain of Xcv controlled by $\underline{Bs_2}$, and HR occurring in pepper to the tomato strain (Cook, 1973; Cook and Stall, 1969). The phenotypes produced by the two reactions in plants inoculated with high concentrations of bacteria are distinguishable from HR controlled by genes $\underline{Bs_1}$ and $\underline{Bs_3}$. Also, both appear to be generalized resistances with no clear evidence for racial differentiation sensu Cook and Stall (1969). Both $\underline{Bs_2}$ and the tomato HR, together with nonhypersensitive resistance controlled by $\underline{Bs_4}$ (or its equivalent) appear to react against the basic complement of genes in Xcv which makes the bacterium a pathogen of pepper. Resistance controlled by genes $\underline{Bs_2}$ and $\underline{Bs_4}$ should prove durable when combined in pepper.

Race differentiation clearly did occur in plants with genes $\underline{Bs_1}$ and $\underline{Bs_3}$ (Cook and Stall, 1969; Kim and Hartmann, 1985; Chapters 3, 4, and 5 of this dissertation). Tests for allelism showed that $\underline{Bs_1}$ is in some but not all plants of both PI lines 163189 and 271322. It was not

located in PI 163192 in which it had first been discovered (Cook and Stall, 1963: 1969; Chapters 3 and 6 of this dissertation). The same allele may occur in few other C. annuum lines (Cook and Stall, 1969). The gene Bs_3 was discovered only in PI 271322 (Kim and Hartmann, 1985). Race 3, which previously had been indistinguishable from race 1 (Cook and Stall, 1969), differed from it by not inducing typical HR in plants with Bs_3 or Bs_1 (Chapter 4, this dissertation). Segregation of Bs_4 in progenies derived from PI 271322 was independent of the dominant HR genes Bs_1, Bs_2, and Bs_3 (Chapters 3, 4, and 5 of this dissertation).

The genes Bs_1 and Bs_3 occurred in pepper germplasm that was nonhypersensitively resistant to races 1, 2, and 3 (Sowell and Dempsey, 1977; Stall, 1981; Chapters 3, 5, and 8 of this dissertation). Resistance controlled by Bs_1 and Bs_3 is therefore basically redundant in these test environments. The origin of these race-specific genes is entirely unknown. The genetic loci in bacteria of races 1 and 2 that are complementary to Bs_1 and Bs_3 are plasmid-borne and appear to be imposed on the complement of genes in the bacterium that cause Xcv to be pathogenic on pepper (Stall, 1985; Stall et al., 1984; R.E. Stall, 1984, personal communication). The plasmid-borne locus in bacteria of race 2 that initiates HR in plants with Bs_1 is linked to one for copper resistance (Stall, 1985). Copper resistance is of selective advantage to the bacterium in Florida agriculture (Marco and Stall, 1983). Plasmids may be introgressed from other bacterial genera by transconjugation (Coplin, 1982; Lai et al., 1977). It is possible that genes Bs_1 and Bs_3 in Capsicum germplasm may mediate response to other phytopathogenic bacteria. These other bacteria may have donated genes to Xcv by natural transconjugation. Once in Xcv, these bacterial loci mediate race specific HR in plants with Bs_1 or Bs_3.

Durable Resistance

The genes $\underline{Bs_1}$ and $\underline{Bs_3}$ control strong and effective resistance to only races 2 and 1 of Xcv, respectively. $\underline{Bs_2}$ and $\underline{Bs_4}$ control race 3. Race 3 occurred as a variant of race 1, and was found among laboratory culture collections. Distribution of race 3 in Florida is not determined but is low in populations because of selection pressure for race 2. Race 3 did not occur among seven isolates sampled in Hawaii (Kim and Hartmann, 1985), nor among four isolates from Queensland (unpublished data). Race 3 did occur when bacteria lost extra-chromosomal plasmids. Loss of plasmid may confer a selective disadvantage on natural populations of Xcv (Stall, 1985). Nevertheless, bacteria of race 3 were virulent on greenhouse-grown peppers in these experiments.

Change of the predominating race may occur very quickly in populations of bacteria challenged by plants with dominant genes for race specific HR (Dahlbeck and Stall, 1979; Dahlbeck et al., 1979). It is possible that selection for virulence will occur in natural populations of races 1 and 2 of Xcv when challenged by plants with both $\underline{Bs_1}$ and $\underline{Bs_3}$, and resistance may be short-lived. Plants with $\underline{Bs_1}$ and $\underline{Bs_3}$ clearly may be highly susceptible to Xcv unless selection for resistance to race 3 is rigorous (Chapters 3, 4, and 5 of this dissertation).

Durability of resistance controlled by $\underline{Bs_2}$ also may not be great. Plants with $\underline{Bs_2}$ have not been challenged extensively with natural populations of Xcv to detect virulent isolates. Genetic recombination within the locus also may occur to expose variability, and environmental factors limiting expression of HR are not known. Nevertheless, resistance controlled by $\underline{Bs_2}$ is highly promising for its broad spectrum

action and ease of selection in segregating populations (Cook, 1977; Chapter 5, this dissertation).

Nonhypersensitive resistance has been noted in the same PI lines over many years and in diverse environments (Adamson and Sowell, 1983; Greenleaf, 1960; Hibberd and Gillespie, 1982a and b; Hibberd et al., 1979; Kim, 1983; Sowell, 1960; Sowell and Dempsey, 1977; Sowell and Langford, 1963; Stall, 1981). However greater effort is required to identify resistant segregates compared with heterozygotes with $\underline{Bs_1}$, $\underline{Bs_2}$ or $\underline{Bs_3}$ (Adamson and Sowell, 1983; Borchers, 1965; Hibberd and Gillespie, 1982a; Chapters 3, 5, 6, 7 and 8 of this dissertation). Selecting segregates by their components of resistance has proved to be the most efficient and reliable technique desscribed to date (Stall, 1981).

The degree of resistance necessary for adequate field control of Xcv may vary with the test environment (Hibberd and Gillespie, 1982b). Nonhypersensitive resistance controlled by $\underline{Bs_4}$ or its equivalent is thoroughly adequate for most environments when in homozygotes (Chapter 8, this dissertation). However, resistance was more complete in inoculated plants when this gene was coupled with $\underline{Bs_1}$, $\underline{Bs_2}$, or $\underline{Bs_3}$ (Sowell and Dempsey, 1977; Chapters 3, 5, 6, and 8 of this dissertation). Stable and durable resistance in PI 271322 was controlled by the complement of genes $\underline{Bs_1}$, $\underline{Bs_3}$, and $\underline{Bs_4}$ (Chapter 3, this dissertation). Breeding resistant peppers should be based on generalized resistance controlled by $\underline{Bs_2}$ or $\underline{Bs_4}$ which if necessary may be supplemented with race specific genes $\underline{Bs_1}$ and $\underline{Bs_3}$.

Unanswered Questions

Resistance controlled by the gene $\underline{Bs_2}$ is the most promising for applied plant breeding in the short term. The gene controls HR to

both races 1 and 3, but irrefutable data linking it also to HR to race 2 is currently lacking. Durability of this resistance also remains unknown, although this may be tested experimentally (Dahlbeck and Stall, 1979). Clear evidence of recombination within the locus also is lacking. Should recombination occur at low frequency, segregates selected only on the basis of HR phenotype will require careful evaluation by several tests to confirm their resistance (Chapter 5, this dissertation).

The distribution in nature of race 3 of Xcv is unknown. Selection for race 3 may occur in plants which have only the genes $\underline{Bs_1}$ and $\underline{Bs_3}$ for HR to races 2 and 1, respectively. However, the loss of conjugative plasmids may reduce the fitness of natural populations of bacteria to such an extent that these two resistance genes, $\underline{Bs_1}$ and $\underline{Bs_3}$, may be adequate for control of bacterial spot in pepper. Selection for race 3 may be unlikely unless populations of bacteria are challenged by genes $\underline{Bs_1}$ and $\underline{Bs_3}$.

Hybrid cultivars with genes $\underline{Bs_1}$, $\underline{Bs_2}$, and $\underline{Bs_3}$ should be easy to breed. These genes are dominant and easily selected by inoculation of cotyledonary or first true leaves. A lower degree of resistance than controlled by these genes may be quite adequate in many field environments (Hibberd and Gillespie, 1982b). Heterozygotes with intermediate resistance controlled by $\underline{Bs_4}$ (or its equivalent) may also be useful releases. Stability of this resistance in heterozygotes has never been examined.

Gene mapping in Capsicum ($\underline{2n} = \underline{2x} = 24$) is very embryonic. Only 22 genes have been mapped by trisomic analysis (E. Pochard, 1984, personal communication). Four chromosomes are still without any mapped

genes. It is desirable that linkage groups be established for genetic studies (Burnham, 1962). Ten of the 12 primary trisomics (Pochard, 1970; E. Pochard, 1984, personal communication) were identified in a small preliminary planting and crossed with a diploid line carrying genes $\underline{Bs_1}$ and $\underline{Bs_3}$. It should be possible to identify with relative ease by trisomic analysis the chromosomes that carry the three genes $\underline{Bs_1}$, $\underline{Bs_2}$ and $\underline{Bs_3}$. These genes control dominant phenotypes that can be identified in young seedlings. More effort will be required to identify the location of $\underline{Bs_4}$ since this resistance is identifiable in mature leaves.

Many questions remain unanswered concerning the comparative physiology of hypersensitive and nonhypersensitive resistance. These include the role of phytoalexins, the environmental limitations to expression of resistance, and ultrastructure of the two forms of resistance in the host.

Race Designation

Three groups of Xcv are differentiated by reaction to inoculation of plants of pepper and tomato (Lycopersicon esculentum Mill.). Isolates of the tomato strain induce HR in pepper and susceptibility in tomato (Cook, 1973; Cook and Stall, 1969). Isolates of the pepper strain are virulent in both pepper and tomato (Cook and Stall, 1969). Kimura et al. (1972) described Brazilian cultures that were virulent on pepper but induced HR in tomato.

Host range is important in the pathovar system of nomenclature (Young et al., 1978). It may be more appropriate to describe three pathovars to replace one (Xcv) following careful verification of the existence of all three groups. Their names may be X.c. pv. lycopersici for the tomato strain, X.c. pv. vesicatoria for the strain pathogenic

on both species (the pepper strain in present terminology), and X.c. pv. capsici for the Brazilian cultures which induce HR in tomato and are virulent on pepper. A pepper plant may be diseased with pathovars vesicatoria and capsici. Similarly, tomato may be diseased with pathovars vesicatoria and lycopersici. This suggested nomenclature is a disadvantage to rapid plant disease diagnosis since host range must be determined. However, distribution of one or more of the suggested pathovars may be so restricted in area that host range determination for all regions may be unnecessary.

Reaction of pepper plants with combination of genes Bs_1, Bs_2, and Bs_3 to inoculation with the suggested pathovar X.c. pv. capsici is entirely unknown. Races of the proposed X.c. pv. vesicatoria (the currently acknowledged pepper strain) are differentiated on pepper plants with combinations of only two genes, Bs_1 and Bs_3 (Chapter 4, this dissertation). Gene nomenclature, which was applied by Kim and Hartmann (1985), followed the terminology of Lippert et al. (1965) in which the genes are subscripted in chronological order of discovery. This has been a source of confusion throughout this study. For example, race 2 induces HR in plants with Bs_1, race 1 induces HR in plants with Bs_3, races 1 and 3 induce HR in plants with Bs_2, and races 1, 2, and 3 are avirulent on plants homozygous for Bs_4 (which controls nonhypersensitive resistance).

It is desirable that race identification be continued in work involving race-specific resistances. An alternative race nomenclature should be made to link genes in both host and bacterium that control race-specific HR. The following nomenclature follows basically the Habgood (1970) system. Advantage may be taken of the biology of the

pathogen in renaming races of pv. vesicatoria. On current evidence, both races 1 and 2 revert directly to race 3. Race 3 appears to contain those genes necessary for basic pathogenicity on C. annuum. This race may be renamed race (0). Race (0) would be virulent on plants with either Bs_1 or Bs_3 (Chapters 3, 4, and 5, this dissertation) but avirulent on plants with Bs_2 (Chapter 5, this dissertation) or Bs_4 (Chapter 3, this dissertation). The currently named race 2 (Cook and Stall, 1969) may be altered to race (1). Race (1) would be avirulent on plants with Bs_1 but virulent on plants with Bs_3 only. The currently named race 1 may be altered to race (3). Race (3) would be avirulent on plants with Bs_3 but virulent on plants with Bs_1 only. A race avirulent on plants with both Bs_1 and Bs_3 would be named race (1,3). Additional races that may be identified in time may be similarly designated. This system may be applied to any named pathovar of X. campestris pathogenic on pepper. It is not applicable to genes Bs_2 and Bs_4, since pathogenic specialization on plants with these genes has not been detected with certainty.

CHAPTER 10
SUMMARY AND CONCLUSIONS

Three races of the pepper strain of Xanthomonas campestris pv. vesicatoria (Doidge, 1920) Dye, 1978 were differentiated in pepper plants with various resistance genotypes involving two independent genes, Bs_1 and Bs_3. Race 1 induces HR in plants with Bs_3, race 2 induces HR in plants with Bs , and race 3 fails to induce HR in plants with either or both Bs_1 and Bs_3. Previously, in the absence of Bs_3, races 1 and 3 were indistinguishable. The HR necroses controlled by Bs_1 amd Bs_3 in the plant were visible within 24 h after inoculation of plants, and the host cell collapse phase of HR was associated with loss of large quantities of electrolytes from inoculated tissues. These parameters are typical for HR, and contrasted with the susceptible reaction in which electrolyte loss from inoculated leaf tissues accumulated slowly.

The gene Bs_1, which was found first in PI 163192 and incorporated into releases of bell pepper, Delray Bell, Florida VR-2, Florida VR-4, and Florida XVR 3-25 occurred also in other PI lines. Tests for allelism involving PI 163189 crossed with Florida VR-4 and PI 271322 crossed with Delray Bell showed that both PI lines had Bs_1. The gene Bs_3 was confirmed to be in only PI 271322.

The times to the cell collapse phase of HR controlled by Bs_1 with race 2 and Bs_3 with race 1 differed consistently. This was observed in inbred plants of a selection, 271-4, from PI 271322, and in hybrid and recurrent backcross progenies of 271-4 crossed with the bell pepper Early Calwonder (ECW). In controlled experiments at 25 and 30 C the times to visible leaf tissue collapse and maximum electrolyte loss from

leaves of 271-4 inoculated with races 1 and 2 were 22 h and 9 h respectively. These differences were consistent with those occurring in greenhouse environments, and attests to the fact that different genes control HR.

Hypersensitivities controlled by $\underline{Bs_1}$ and $\underline{Bs_3}$ in plants of 271-4 were incompletely dominant, i.e., time to visible host tissue collapse and maximum electrolyte loss at 25 C occurred less rapidly in hybrids of 271-4 and ECW than in 271-4 homozygotes. The delay in time in heterozygotes was 4 to 5 h for $\underline{Bs_1}$, and 2 to 3 h for $\underline{Bs_3}$. Maternal effects were absent. Incomplete dominance of the genes was also noted with bacterial populations *in vivo* in greenhouse-grown plants inoculated with 2×10^3 cfu ml^{-1}. Dominance for bacterial populations was greater for $\underline{Bs_3}$ than for $\underline{Bs_1}$. Nevertheless, penetrance of both $\underline{Bs_1}$ and $\underline{Bs_3}$ was great in heterozygotes. Both genes may be regarded as dominant for the purposes of applied plant breeding.

The two genes, $\underline{Bs_1}$ and $\underline{Bs_3}$, segregated independently in breeding progenies (including second and third backcrosses) derived from crossing 271-4 with ECW. Heterozygotes for both genes were identified with greater than 98.5% accuracy by inoculating cotyledonary leaves that recently had become fully expanded. The error rate, which was determined by subsequently inoculating mature leaves, was sufficiently low that inoculating young seedlings is recommended. Populations may now be grown in seedling flats and rapidly screened for both genes in less time than previously required.

The bell pepper Florida XVR 3-25 had been selected for the genes $\underline{Bs_1}$ and $\underline{Bs_2}$. The gene $\underline{Bs_2}$ controlled HR to race 1. Florida XVR 3-25

was found to be hypersensitive to all three races of Xcv. Hypersensitivity was confirmed by examining time to host tissue collapse and maximum electrolyte loss from leaf tissues inoculated with 5×10^8 cfu ml^{-1} of bacteria of each race. Race 2 induced the most rapid HR, and time to reaction was shorter at 30 than 25 C. The reactions to races 1 and 3 did not differ, and developed faster at 25 than 30 C. These differences are further evidence that the expression of HR varies with the controlling gene.

A test for allelism confirmed that both Florida XVR 3-25 and 271-4 (from PI 271322) carried Bs_1. The genes Bs_2 and Bs_3, which were different loci, segregated independently, and each controlled phenotypically distinguishable HR to race 1. The HR necrosis controlled by Bs_2 was characteristically brown and incompletely desiccated, while that controlled by Bs_3 was completely desiccated and gray-white in color. The gene Bs_2 in Florida XVR 3-25 is unique in that it also simultaneously controls HR to race 3. The parents crossed for this test precluded examining the added hypothesis that Bs_2, like Bs_1, controls HR also to race 2. Subject to confirmation of this hypothesis, gene Bs_2 may be a rare example of a resistance gene that combines all the desirable attributes, namely, it is monogenic and dominant, and controls a major effect that is both easily recognized in young plants and generalized against all races of the pathogen.

Notwithstanding the different physiologies of HR controlled by genes Bs_1, Bs_2, and Bs_3, the net effect in homozygotes or heterozygotes was the same, that is, populations of bacteria *in vivo* were vastly lower than in susceptible plants. The difference in populations of bacteria between susceptible plants and plants homozygous for Bs_1 and Bs_3 was a

factor of 10^4 to 10^5. This difference was reflected in low disease severity in inoculated leaves of plants with $\underline{Bs_1}$ or $\underline{Bs_3}$.

Resistance to race 3 of Xcv occurred in all 18 PI lines evaluated. This form of resistance was generally unlike HR controlled by $\underline{Bs_1}$, $\underline{Bs_2}$, and $\underline{Bs_3}$, that is, it was quantitative and not qualitative. For these studies, this resistance was termed nonhypersensitive. Nonhypersensitive resistance was evaluated 2 to 3 weeks after inoculation of leaves with low concentrations of bacteria (i.e., approximately 2×10^3 cfu ml^{-1}). The components of resistance were measured, namely, the number of lesions per unit area of leaf and the diameter per lesion. Low populations of bacteria *in vivo* in inbred plants of five PI lines (163189, 173877, 244670, 246331, and 271322) were strongly correlated with low values of both components of nonhypersensitive resistance.

Nonhypersensitive resistance to multiplication of bacteria of race 3 in selection 271-4 of PI 271322, and in heterozygotes of 271-4 crossed with ECW, became effective by about 4 to 5 days after inoculation with low concentrations of bacteria of race 3. This contrasted with 2 days required for induction of HR to races 1 and 2 in the same plants. Multiplication of bacteria of race 3 in heterozygotes was less extensive than in ECW but greater than with races 1 and 2 in the same plants.

All 18 PI lines tested were highly resistant in a replicated field planting. Field resistance was highly correlated with the two components of resistance (lesions per unit area of leaf and diameter per lesion) measured on the same lines in a replicated greenhouse experiment. However, of the two components, lesion diameter was clearly the more strongly correlated with field resistance. This was taken to reflect lower rates of bacterial multiplication *in vivo* more accurately than lesion numbers per unit area of leaf.

Inheritance of nonhypersensitive resistance was evaluated in progenies (F_1, F_2, and backcrosses) of inbred selections of each of three PI lines (163189, 246331, and 271322) crossed with susceptible bell peppers. In all cases, nonhypersensitive resistance controlled races 1 and 2 in addition to race 3. The degree of resistance was not directly related to the race of Xcv, but varied with the aggressiveness of the bacterial isolates. Resistance was greatest when plants of all generations were inoculated with the weakest isolates, and least with the most aggressive isolates.

In all studies, inheritance of nonhypersensitive resistance was consistent with single factor, additive gene action controlling smaller lesions. Resistance to lesion expansion was so great in heterozygotes inoculated with weak isolates that relatively few necrotic areas per unit area of leaf became visible lesions. These lesions were of diameter approximately intermediate to those of both parents. In contrast, expansion of lesions of aggressive isolates was sufficient for all necrotic areas to become visible lesions in heterozygotes. These lesions however were also of diameter intermediate to both parents.

The proposed symbol for the additive gene for nonhypersensitive resistance from PI 271322 is $\underline{Bs_4}$. Tests for allelism of $\underline{Bs_4}$ with loci in PI 163189 and PI 246331 were not carried out. The gene $\underline{Bs_4}$ along with $\underline{Bs_1}$ and $\underline{Bs_3}$, were transferred by selection to advanced recurrent backcrosses with a bell pepper background.

An array of genes controlling resistance to bacterial spot now exists in released bell peppers and advanced breeding lines. The prospects for their commerical usage are particulary encouraging.

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BIOGRAPHICAL SKETCH

Allen Maxwell Hibberd is native to Queensland, Australia. His birth date was October 5, 1950. Primary and secondary education was received in public schools in Brisbane, Queensland. The degree of Bachelor of Agricultural Science with Honours was earned at the University of Queensland in 1971. His major was quantitative genetics and plant breeding. He completed almost eleven years employment as Horticulturist with the Queensland Department of Primary Industries who, in conjunction with the Vegetable Crops Department of the University of Florida and the Committee of Direction of Fruit Marketing of Queensland provided support for the present study from August 1982. Upon return to Queensland, he will resume duties as Horticulturist at the Redlands Horticultural Research Station, Ormiston. It is his hope that the materials to be forthcoming from the present study will mutually benefit both Queensland and Florida.

He and his wife Jo-Anne (nee Homann) of twelve years have been blessed with three children Anthony Noel, Kerri Anne, and Allison Kathleen.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Mark J. Bassett, Chairman
Professor of Horticultural Science

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